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MELBOURNE

THE EFFECT OF ASSOCIATE PERENNIAL GRASSES ON A SIMPLE PASTURE MIXTURE OF PHALARIS AND SUBTERRANEAN CLOVER

By W. D. ANDREW* and C. A. NEAL-SMITH*

(*Manuscript received February* 24, 1956)

Summary

Over the period 1952-1954 there was no significant difference in the yield of herbage produced annually by the addition to a *Phalaris tuberosa* L.–*Trifolium subterraneum* L. pasture mixture, of any one of the following grasses: *Agropyron obtusiusculum* Lange., *Bromus coloratus* Steud., *Bromus inermis* Leyss., *Dactylis glomerata* L., or *Festuca arundinacea* Schreb.

There were indications of a small change in seasonal production where certain grasses, notably *D. glomerata*, were included in the mixture.

Over the 3-year period the proportion of the sown grass component, in the mixtures where either *D. glomerata*, *B. inermis*, or *B. coloratus* were included, increased by a greater amount than where the simple mixture of phalaris and subterranean clover was used. The addition of each grass also lessened the amount of *P. tuberosa* in the sown grass component of the yield.

In the third year, despite the varying proportions of the phalaris and associated sown grass species, the mean population of the sown perennial grasses in each treatment did not differ significantly from the mean figure of 1.34 plants/sq. lk.

The increased production of the sown grass yield component following the association of certain of the above species with *P. tuberosa* suggests that the latter does not fully exploit the environment.

The principle of including another perennial grass when sowing phalaris and subterranean clover might have wide application as a means of combatting "phalaris staggers".

I. INTRODUCTION

Sown pasture development in Australia under natural rainfall conditions is now based largely on the use of simple mixtures, quite frequently of only one grass and one legume species. This is in marked contrast to the old practice of sowing complex mixtures, which prior to 1929 often included as many as 12 or 14 species, as pointed out by Harrison (1936). When such complex mixtures were sown, many of the species failed to survive or else made an insignificant contribution to the total herbage yield (Moodie 1931).

The general recognition, therefore, of the negligible contribution of many species in such mixtures led to the trend towards simplification, but it is possible that the change in this direction may have been too extreme.

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In the southern part of Australia a simple pasture mixture of phalaris (*Phalaris tuberosa* L.) and subterranean clover (*Trifolium subterraneum* L.) is commonly grown as pasturage for sheep and cattle. Although these species have a similar growth rhythm and produce very little during the summer and winter periods, this type of pasture is generally considered satisfactory and it is widely believed that its productivity is all that could be desired. There is, however, no published evidence that these two species fully exploit the environment or that other sown grasses could not be grown in association and add further to the yield or change the growth rhythm to advantage or both; although the possibility of improvement through the use of associated grasses may have been implied by Whittet (1935) when he recommended the admixture of other grasses with phalaris for sown pastures in certain districts of New South Wales.

Because of the disorder known as "phalaris staggers" which occurs in certain seasons when phalaris is the only grass available to the stock (Cook *et al.* 1953), it would seem advisable to have other grasses in the mixture to reduce the proportion of phalaris at these times.

The experiment described herein was undertaken to study the changes in botanical composition and productivity which followed when certain grasses known to be capable of vigorous growth in the Canberra environment were added to a simple mixture of phalaris and subterranean clover.

II. Experimental Procedure

The project was carried out at the Dickson Experiment Station, Canberra, A.C.T., from 1952 until 1954 inclusive. It was located on podzolized grey to brown loam with a pH of about 5.8 which had previously grown red clover (*Trifolium pratense* L.) for 2 years immediately before sowing down to the pasture plots.

The six treatments were (number of germinable seed per sq. lk sown is shown in brackets):

(1) "Control" *Phalaris tuberosa* L. (6.0) + *Trifolium subterraneum* L., strain Bacchus Marsh (3.0).

(2) *P. tuberosa* (6.0) + *Bromus coloratus* Steud. (6.0) + *T. subterraneum* (3.0).

(3) *P. tuberosa* (6.0) + *Festuca arundinacea* Schreb., strain Kentucky 31 (9.0) + *T. subterraneum* (3.0).

(4) *P. tuberosa* (6.0) + *Bromus inermis* Leyss. (9.0) + *T. subterraneum* (3.0).

(5) *P. tuberosa* (6.0) + *Dactylis glomerata* L., strain Gippsland (9.0) + *T. subterraneum* (3.0).

(6) *P. tuberosa* (6.0) + *Agropyron obtusiusculum* Lange. (6.7) + *T. subterraneum* (3.0).

The origin of the species listed above is as follows:

P. tuberosa	Australian commercial seed
D. glomerata	Australian commercial seed
T. subterraneum	Australian commercial seed
A. obtusiusculum	C.P.I.* 9353 — Canada
B. coloratus	C.P.I. 9709 — Uruguay
B. inermis	C.P.I. 7073 — U.S.A.
F. arundinacea	C.P.I. 10339 — U.S.A.

Seeding rates were calculated with the objective of obtaining an establishment of 2 plants/sq. lk in the case of phalaris and subterranean clover, and 3 plants/sq. lk in the case of the other species.

The individual plots were 25 by 80 lk and the six treatments were laid out in the form of a Latin square.

Sowing was carried out by hand-broadcasting of seed on April 22, 1952. Fertilizer applications were: (1) immediately prior to sowing, 5 cwt carbonate of lime and 5 cwt superphosphate per acre applied with a drill; (2) in 1953, 3 cwt superphosphate, 2 oz ammonium molybdate, and 3½ lb borax per acre; (3) in 1954, 2 cwt superphosphate per acre.

Seedlings were counted on July 9, 1952, to determine the density of establishment, and in the autumn of 1954 plant counts were again made to ascertain the survival of the sown grasses. In addition one replicate was counted in July 1953.

Throughout the trial, yields were determined by cutting appropriate numbers of quadrat areas to near ground level, hand-sorting subsamples to determine the botanical composition of the herbage, and then weighing the oven-dried material to determine yields.

During the first year, sampling was carried out at the period of maximum growth in the springtime. In 1953 the amount of growth during the autumn, winter, and spring periods was separately determined. In the third year of the trial, summer and autumn production was measured, and then because of the poor winter growth only the combined winter and spring growths were determined.

After each sampling the pasture was quickly grazed down with sheep to the same level as the defoliated quadrat sampled areas and was then left ungrazed until the next sampling was carried out.

The monthly rainfall and mean temperatures during the period of the trial are shown in Table 1. It will be observed that the rainfall in 1952 was substantially above the average, which favoured establishment of sown species. In the following two years it was below average, particularly in 1954. Throughout the duration of the trial there were no great departures from the normal mean monthly temperatures of this environment.

* Commonwealth Plant Introduction.

III. Results

(*a*) *Productivity of Pasture*

The yields of herbage, including sown and miscellaneous species, obtained over the 3 years are shown in Figure 1.

It will be noted that although phalaris never became highly productive it did become progressively more vigorous with time. This is typical of the behaviour of phalaris when sown in a pasture mixture.

Table 1

MONTHLY RAINFALL AND MEAN TEMPERATURES AT DICKSON EXPERIMENT STATION, CANBERRA, A.C.T., FOR THE PERIOD 1952-1954

Month	1952		1953		1954		Mean 1942-54	Mean 1942-54
	Rainfall (in.)	Temp. (°F)	Rainfall (in.)	Temp. (°F)	Rainfall (in.)	Temp. (°F)	Rainfall (in.)	Temp. (°F)
Jan.	1.19	71	1.69	65	1.97	68	1.79	68
Feb.	0.46	67	0.43	65	2.27	66	1.99	67
Mar.	5.10	64	0.98	62	0.01	61	2.16	62
Apr.	5.14	56	0.92	56	1.58	58	1.92	55
May	2.75	46	6.18	48	0.42	47	2.72	47
June	4.68	44	0.96	41	1.50	42	1.66	43
July	1.58	40	0.51	40	0.71	42	1.49	41
Aug.	2.38	44	1.02	43	1.13	46	1.54	44
Sept.	2.81	47	1.89	48	0.58	48	1.63	49
Oct.	4.93	52	1.53	52	1.69	56	2.03	53
Nov.	2.17	54	1.94	56	3.40	61	2.69	59
Dec.	4.28	61	1.45	65	1.32	66	2.06	64
Total	37.47		19.50		16.58		23.68	

On the other hand subterranean clover, which grew very vigorously in the first year, was only moderately productive in the second year and virtually failed in the third year. This is not considered to be the direct result of competition from other species, *per se*, for reference to the rainfall figures for the autumn and winter will indicate that the distribution in 1953 and particularly in 1954 was unfavourable for this species.

With the exception of *F. arundinacea* the other sown grasses became progressively more productive with time.

The histograms also show that the addition of an extra grass to the mixture did not bring about any great change in the annual production of herbage and, apart from the autumn of 1953 when late summer rains stimulated a stronger autumn growth of grass than is usual, it did not change seasonal production of herbage to any significant degree. In the autumn of this particular year, however, the productivity of the sown grass component in those mixtures containing *B. coloratus* or cocksfoot (*D. glomerata*) was greater than the control.

(*b*) *Botanical Composition of the Pasture*

Very marked differences in botanical composition developed between the various pasture mixtures, owing to differences in both numbers and productivity of individual plant species, and these differences became more pronounced with time.

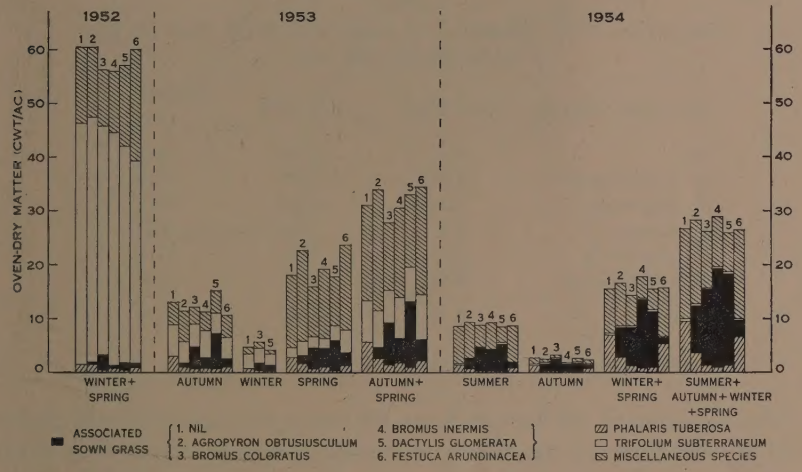

Fig. 1.—Yield and botanical composition of herbage for each sampling during the period 1952-1954.

The sown grass component of the various mixtures became progressively more vigorous and productive, and this was coupled with a corresponding reduction in the yield of the miscellaneous unsown fraction.

TABLE 2

PERCENTAGE CONTRIBUTION BY *P. TUBEROSA* TO TOTAL SOWN GRASS COMPONENT OF ANNUAL YIELD

Associate Species	1952	1953	1954
Nil	100.0	100.0	100.0
A. obtusiusculum	85.5	55.0	33.4
B. coloratus	13.4	15.4	11.7
B. inermis	71.0	29.3	15.8
D. glomerata	21.6	17.0	8.1
F. arundinacea	61.7	36.3	77.8
Mean of 5 treatments	50.6	30.6	29.3

After the first year the contribution from subterranean clover on all treatments declined and was very poor in 1954, when the seedlings did not establish until the early winter.

Whilst during the first year the production from the "total sown grass" component of the mixture showed comparative uniformity and differences between treatments were not significant, in the second and third years as the sown grasses became stronger marked differences in both yield and composition became evident.

TABLE 3

POPULATION OF *P. TUBEROSA* IN FIRST AND THIRD YEAR OF THE TRIAL (PLANTS/SQ. LK)

Associate Species	9.vii.1952	1.iii.1954	Reduction (%)
Nil	2.97	1.12	62.3
A. obtusiusculum	2.87	0.59	79.4
B. coloratus	2.24	0.22	90.2
B. inermis	2.74	0.29	89.4
D. glomerata	3.08	0.09	97.1
F. arundinacea	2.65	0.73	72.4
Mean	2.76	0.51	81.5
Min. sig. diff. ($P = 0.05$)	N.S.	0.28	

The inclusion of the various sown grasses generally depressed phalaris but in at least three of the mixtures, namely, those in which *B. coloratus*, *B. inermis*, and *D. glomerata* were sown, the production of each of these species in both the second and third years more than compensated for the reduction in yield of phalaris.

TABLE 4

YIELD OF *P. TUBEROSA* ON AN INDIVIDUAL PLANT BASIS FOR THE AUTUMN OF 1954, SOWN ALONE AND WITH VARIOUS ASSOCIATE GRASSES

Associate Species	Yield per Plant (g)	Percentage of Control
Nil	0.54	100
A. obtusiusculum	0.28	52
B. coloratus	0.24	44
B. inermis	0.24	44
D. glomerata	0.23	43
F. arundinacea	0.58	107

The change in composition is shown by the decreasing contribution by phalaris with time on all treatments except where the associated grass was *F. arundinacea*, which had lost vigour by the third year (Table 2).

This decrease in the contribution from phalaris was associated with both a decline in population (Table 3) and a lower productivity of individual plants (Table 4).

The greatest reduction in population and vigour of the phalaris occurred when *D. glomerata* was the associate grass, this being the most productive of the other sown grasses over the preceding 2-year period.

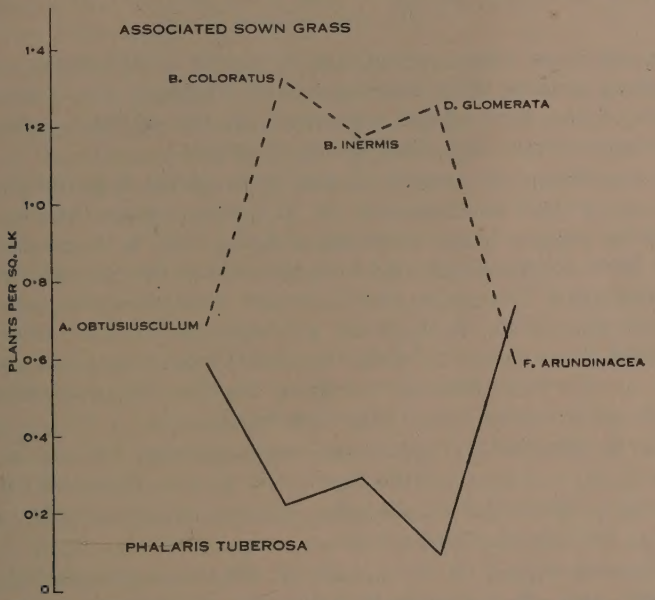

Fig. 2.—The inverse relationship between the populations of *P. tuberosa* and associated sown grasses at March 1, 1954.

TABLE 5

POPULATION DENSITIES OF TOTAL SOWN PERENNIAL GRASSES AT BEGINNING AND END OF TRIAL (PLANTS/SQ. LK)

Associate Species	First Year (as seedlings) 9.vii.1952	Third Year (as mature plants) 1.iii.1954
Nil	2.97	1.12
A. obtusiusculum	6.28	1.28
B. coloratus	7.74*	1.54
B. inermis	5.52	1.46
D. glomerata	6.15	1.34
F. arundinacea	5.28	1.31
Min. sig. diff. ($P = 0.05$)	0.13	N.S.

* Includes some *Bromus sterilis* L.

An inverse relationship between the population densities of phalaris and of the associated sown grass is clearly shown in Figure 2. In Table 5

it will be seen that the total population of sown grasses tends toward a constant level in the third year, whereas there were marked differences in the year of sowing.

IV. Discussion

Although there was no significant change in total herbage production when various grasses were associated with phalaris, the increase in the proportion of the sown grass component in the mixture indicates that phalaris was not fully exploiting the environment.

The association of certain grasses with phalaris permitted greater exploitation of the environment by the sown grass species because conditions at certain times were more favourable to them than to the phalaris. Hence in the autumn and winter of 1953 the mixtures containing *B. coloratus* and *D. glomerata* had a greater yield of sown grass than the control, but during the springtime, although the *D. glomerata* mixture still retained its superiority both in yield of sown grasses and in the reduction of the miscellaneous fraction, the *B. coloratus* mixture just failed to be significantly better than the control.

Again in 1954, the *D. glomerata* mixture with its reduced miscellaneous fraction was superior throughout the year in botanical composition to the control, while the *B. coloratus* mixture was similarly better but only during the summer and autumn.

The growth vigour of *B. inermis* in 1954 was considerably greater than in the preceding year and hence the mixture in which it was included was superior to the control in terms of the yield of its sown grass component and its lessened miscellaneous fraction.

This effect was evident at all times of the year but especially in the springtime, when the late October rainfall was more favourable to the *B. inermis* than to the associated phalaris, and at this time the yield of the sown grass fraction in the former mixture was almost double that of the control.

These changes in the botanical composition of the herbage are quite significant and important. The increased proportion of the sown grass component, coupled with a decrease in the unsown miscellaneous fraction, gave better quality in the herbage for grazing if it is accepted that sown grasses are more nutritious than volunteer annual grass species and other weeds.

That this change can be brought about without loss of total production of herbage, and possibly with an increase in its quality, indicates one means of using such species as phalaris and subterranean clover without the risk of phalaris dominance and phalaris staggers.

At the end of 3 years the population of phalaris in the control treatment had reached dynamic equilibrium with the environment. The density at this time was 1.12 plants/sq. lk, which is approximately the

population density found in older phalaris-subterranean clover pastures at the Dickson Experiment Station (unpublished data). The fact that the total sown grass populations in the other treatments were of the same order suggests that they too were close to equilibrium so far as population density was concerned.

It is not possible to predict from the data how long each individual grass would persist beyond a 3-year period but there is evidence that the long succession of dry months during 1954 has adversely affected the survival of *B. coloratus* and *D. glomerata* into the fourth year. However, in a previous trial at Canberra of two of the species, namely, *D. glomerata* (Akaroa strain) and a strain of *F. arundinacea* (previously called *F. elatior*), over a period of 2½ years under both regular cutting and grazing treatments, the former species was found by McTaggart (1935) to possess good powers of persistence and ability to withstand competition. With the latter, its longevity appeared to be in doubt under grazing owing, it was suggested, to its relatively high palatability compared with phalaris and its consequent inability to compete with this species.

A strain of *B. inermis* (C.P.I. 1297) was reported by McTaggart (1942) to be very persistent and productive in both the Southern and Northern Tableland areas of New South Wales.

When this present trial was concluded *A. obtusiusculum* was building up into a dense sward owing to its rhizomatous habit and it was clear that its productivity was on the increase, whereas *F. arundinacea,* although present at a population density of 0.58 plants/sq. lk, was making little contribution to the mixture.

It is evident from this experiment that a significant improvement can be effected by sowing either *B. coloratus, B. inermis,* or *D. glomerata* with phalaris and subterranean clover, but it is thought that the rates of seeding used in this trial were too high for best results.

It is suggested that the principle of sowing another grass with phalaris might well be applied in other environments where phalaris is grown, the particular perennial grass to be used depending upon the prevailing conditions.

V. Acknowledgments

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EFFECT OF INTENSITY OF DEFOLIATION ON REGROWTH OF PASTURE

By R. W. BROUGHAM*

(*Manuscript received March* 1, 1956)

Summary

A pasture association comprising short-rotation ryegrass, red clover, and white clover was subjected to three different intensities of defoliation by cutting down to 1, 3, and 5 in.

At 4-day intervals over a period of 32 days, measurements were taken of herbage dry matter yield, the leaf area per unit area of ground, and the percentage of light penetrating to a level 1 in. above the ground surface.

Where pasture was defoliated to 1 in., light interception was almost complete (95 per cent. or over) approximately 24 days after cutting, whereas pastures defoliated to 3 in. and 5 in. intercepted almost all the incident light 16 and 4 days after cutting respectively.

At these stages of growth the leaf area was approximately 5 sq. ft per sq. ft of ground and the herbage yield approximately 1450 lb dry matter per acre, regardless of treatment.

The rate of pasture growth increased until complete light interception was approached, and thereafter an almost constant maximum rate was sustained.

Leaf efficiency (the rate of increase of herbage dry weight per unit area of leaf) was greatly influenced by intensity of defoliation. Efficiency was initially lower following severe defoliation than following less severe treatment. It increased rapidly to a maximum and thereafter declined gradually. Maximum efficiency in the 3 in. and 5 in. cutting treatments was attained when maximum growth rate was first reached. For pasture defoliated to 1 in., it reached a maximum level during the phase of accelerating growth.

I. INTRODUCTION

In a previous study (Brougham 1955) the nature of the growth curve of a short-rotation ryegrass (*Lolium perenne* × *L. multiflorum*), red clover (*Trifolium pratense*), and white clover (*Trifolium repens*) pasture following close defoliation was determined. This curve of growth showed three distinct phases. For approximately 3 weeks following defoliation the growth rate increased, then for 5 weeks it was constant, and thereafter it declined.

The results obtained from the above study suggested that the duration of the first phase of growth was related to leaf area and light interception, and that this growth phase continued until there was sufficient leaf area to intercept all the incident light. The present study was undertaken to test this assumption by determining the influence of intensity of defoliation on subsequent rate of growth and light interception.

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The literature dealing with the effects of intensity and frequency of defoliation on the growth of pasture plants and associations has been reviewed by Donald (1941) and Wagner (1952). In general the results have shown that as the intensity of defoliation increases, yield of shoot tissue decreases. Similar results have been obtained by varying the frequency of defoliation, the greatest reductions in yield having occurred where the defoliations have been most frequent.

In previous studies in which pasture associations have been investigated, differences in the rate of regrowth have usually been explained in terms of the quantity and the parts of the shoot growth removed and the effect on subsequent photosynthetic activity, and the quantity of stored reserves in the plant. No direct experimental evidence is available, however, on the rate of regrowth of pasture in relation to leaf area and the capacity of the pasture to intercept light.

II. Experimental Methods

A field plot experiment was carried out at the Grasslands Division, Palmerston North, on Manawatu silt loam. The pasture was a short-rotation ryegrass–red clover–white clover association in its third year from sowing. For 2 years before the commencement of the experiment it had been grazed with sheep whenever the herbage reached a height of 6-9 in. When the experiment started, on September 16, 1954, the grass : clover ratio was approximately 75 : 25.

At the commencement of the experiment the pasture was approximately 9 in. high. Areas were cut back to heights of 1, 3, and 5 in. using a sickle bar mower, fitted with adjustable outriggers to control the cutting height. Each treatment was replicated three times in a Latin-square layout. The nine areas were each 32 by 15 ft and were surrounded by mown headlands. Each area was divided into eight plots, each 4 by 15 ft.

Regrowth was measured over a 32-day period from defoliation, every fourth day one randomly sited plot in each area being cut. Each plot was sampled only once during the experiment. Measurements of herbage production, botanical composition, the percentage of light penetrating to a level 1 in. above the ground, and leaf area per unit area of ground were taken.

Herbage yields from the 1 in. treatment were determined as in a previous experiment (Brougham 1955). To determine the increment in herbage from the time of defoliation in the 3 in. and 5 in. treatments, the quantity of herbage left above the 1 in. level at the initial defoliation was measured by mowing parts of the experimental area alongside each block reserved for headlands, first to 3 or 5 in. and then to 1 in. The dry weights of the herbage from these second cuts were then subtracted from

subsequent yield measurements which were obtained by cutting to 1 in. The procedure was adopted so that any new growth that occurred below 3 in. and 5 in. for the 3 in. and 5 in. treatments respectively would be measured. The practical difficulties of lodging from wind-blow were also eliminated.

Determinations of botanical composition were obtained as in the previous experiment (Brougham 1955).

The percentage of light that penetrated to a level 1 in. above the ground surface was measured using an instrument constructed by the Dominion Physical Laboratory, D.S.I.R., Wellington. This instrument comprised two photocells surrounded by diffusing screens approximately 2 in. long and 1 in. wide, each housed in cylindrical tubes 1 in. in diameter and 1 ft in length and connected to independent portable leads. Each photocell recorded on a separate discharge counter through a condenser, and the two photocells were so adjusted that in the same light and in a horizontal plane the frequency of discharge of one was 10 times as great as that of the other. A check reading was taken with both photocells exposed to full light. Then, after placing one of the photocells 1 in. above ground level in the pasture, a second reading was made and the percentage of light penetrating to the 1 in. level was calculated. For each measurement the recording head was pushed horizontally into the sward at ground level without disturbing the herbage. At each sampling date six determinations were made within each plot to be cut, so that at every sampling 18 recordings were obtained for each treatment.

Leaf area of ryegrass and clover was determined from two randomly sited sq. ft quadrats in each plot. The herbage in each quadrat was cut to ground level, and separated into ryegrass and clover and further into leaf and stem. Only the laminae were classed as leaf. Petioles and leaf sheath were classed as stem. Forty ryegrass leaves were taken at random and the mean width of central 100 mm sections determined. These sections were then oven-dried and the weight per unit area calculated. The remainder of the ryegrass leaf obtained from the quadrat was also oven-dried and weighed, and the area of ryegrass leaf per sq. ft of ground was calculated. The area of clover leaf was similarly determined using disks of known diameter taken from leaves. The measurements of leaf area were made for one surface only.

Yield measurements are expressed throughout as lb dry matter per acre, and leaf area measurements as leaf area per sq. ft of ground.

III. Results

Table 1 shows the mean measurements of total herbage, ryegrass, and clover yields, percentage light interception at a level 1 in. above the ground surface, and leaf area of the pasture per sq. ft of ground.

TABLE 1

MEAN YIELDS OF RYEGRASS, CLOVER, AND TOTAL HERBAGE AT EACH SAMPLING DATE TOGETHER WITH LEAF AREA AND PERCENTAGE LIGHT PENETRATION FOR EACH TREATMENT

Yields are given in lb dry matter per acre. Yields of unknown species were negligible

Measurement	Height of Defoliation (in.)	Herbage Remaining after Defoliation	Days of Growth							
			4	8	12	16	20	24	28	32
Mean yield:										
Ryegrass	1	—	85	174	335	550	734	1055	1386	1731
	3	332	430	590	780	969	1337	1561	1982	2428
	5	836	1043	1382	1495	1860	1927	2515	2971	3191
Clover	1	—	20	35	97	200	270	400	650	675
	3	90	144	199	314	455	490	724	737	700
	5	305	389	364	628	664	693	780	760	969
Total herbage	1	—	106	210	435	755	1019	1460	2048	2424
	3	424	578	799	1100	1447	1845	2296	2784	3163
	5	1146	1441	1770	2162	2547	2655	3319	3756	4199
Percentage light interception 1 in. above ground	1	—	12.7	46.3	72.1	86.5	92.1	97.5	98.8	99.1
	3	—	71.6	83.4	89.5	96.5	97.0	98.9	99.1	99.3
	5	—	96.5	97.5	98.9	98.9	98.8	99.3	99.5	99.5
Leaf area (sq. ft of leaf per sq. ft of ground)	1	—	1.74	2.16	2.45	3.52	3.86	4.80	5.36	6.81
	3	—	3.54	5.12	4.80	5.06	5.18	5.84	6.81	7.78
	5	—	5.42	6.55	7.16	7.36	8.01	8.39	8.93	7.60

(*a*) *Light Intensity Measurements*

The results presented in Figure 1 show the percentage of incident light intercepted by plant tissue above the 1 in. level in relation to the

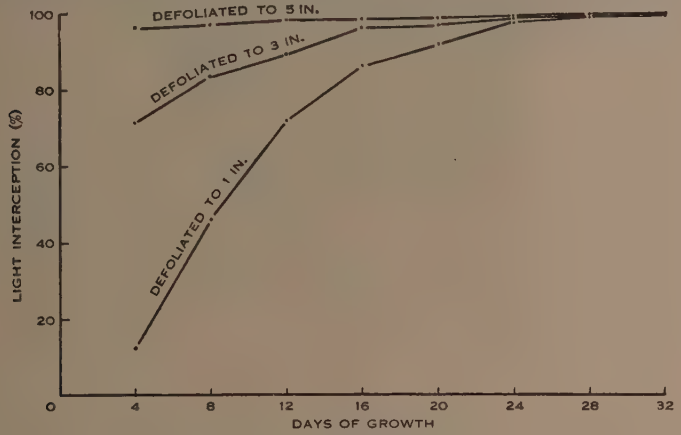

Fig. 1.—The percentage of light intercepted by the herbage above the 1 in. level.

number of days of growth. The plotted points are means of 18 measurements.

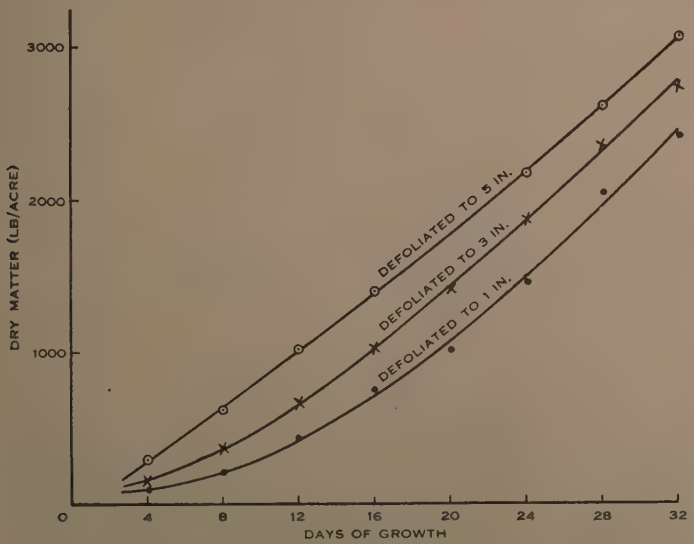

Fig. 2.—Growth curves of total herbage.

(*b*) *Dry Matter Yields*

Figure 2 shows the total herbage yields for the three treatments plotted against time.

In order to smooth the measurement errors and allow yield and increment relationships to be examined, polynomials have been fitted. These give more efficient estimates of increments and their changes than the raw data. As the cubic was the highest significant order met (in the 3 in. treatment) this was fitted throughout. The value at 20 days in the 5 in. treatment was omitted since by testing the deviations of the recorded values from those given by fitting the cubic equation it was found that this value was a significantly outlying observation. The average standard errors of estimate are $\pm$ 61, $\pm$ 23, and $\pm$ 17 lb dry matter per acre for the 1 in., 3 in., and 5 in. treatments respectively.

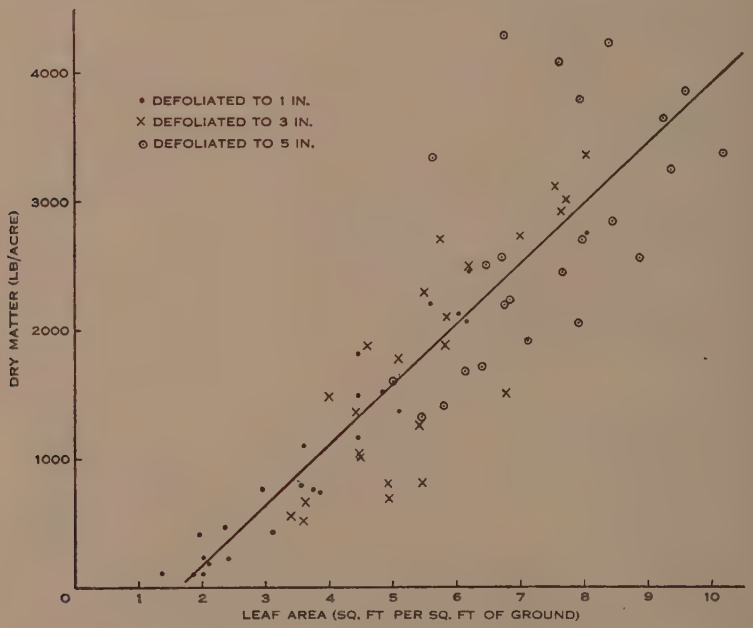

Fig. 3.—The relationship between leaf area and herbage yield.

(*c*) *Leaf Area Measurements*

Figure 3 shows the relationship between leaf area and herbage yields for the three treatments.

An overall regression line only is plotted, as regression lines fitted to the treatments separately were neither significantly curvilinear nor of differing slopes.

The overall regression coefficient and its standard error were 468 $\pm$ 28, the interpretation being that an increase of 1 sq. ft. of leaf area was accompanied by an increase in herbage yield of 468 lb dry matter per acre over the range of these data.

The percentage of leaf in the 3 in. and 5 in. treatments changed little with time and ranged from 53 to 61 per cent. of the total herbage. In the 1 in. treatment the range was greater and the percentage was initially low following defoliation (49.7 per cent.). It then increased to a maximum value (68.1 per cent.) 24 days after defoliation and thereafter decreased.

(*d*) *Leaf Efficiency Determinations*

Leaf efficiency indices were calculated for each sampling date as

(Average daily herbage dry matter increment/Area of leaf).

This term has been preferred to that of net assimilation rate, because the latter has been used in different senses by other workers, as pointed out by Williams (1946), Watson (1952), Mitchell (1954), and others.

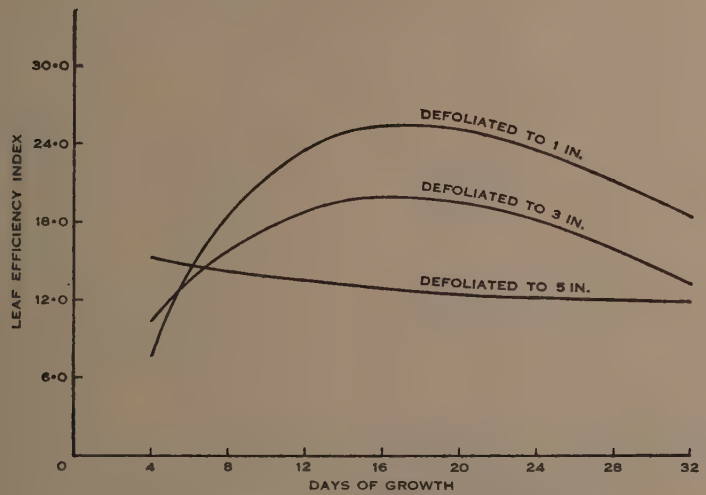

Fig. 4.—Leaf efficiency at various stages of regrowth.

Figure 4 shows changes in leaf efficiency during the regrowth of pasture.

The leaf efficiency indices $(dy/dt)/A$, where dy/dt is the rate of increase of herbage yield and A is the total leaf area in sq. ft per sq. ft of ground, were obtained from values of dy/dt and A taken from smoothed curves of herbage yield (Fig. 2), and leaf area plotted against time (not presented here). Only a first degree regression line could be fitted to the leaf area data for the 5 in. treatment, so that the leaf efficiency curve given for this treatment may be over-simplified. The standard errors of estimate are $\pm$ 1.7, $\pm$ 0.9, and $\pm$ 0.7 for the 1 in., 3 in., and 5 in. treatments respectively.

IV. Discussion

Where the initial defoliation height was 1 in. no light was at first intercepted but with subsequent growth an increasing proportion of light was intercepted. Light interception was almost complete (95 per cent. or over) approximately 24 days after cutting (Table 1 and Fig. 1).

Where the pasture was cut down initially to 3 in. complete light interception was obtained 16 days after cutting but where the initial cutting height was 5 in., sufficient herbage was left to intercept nearly all the incident light immediately following cutting.

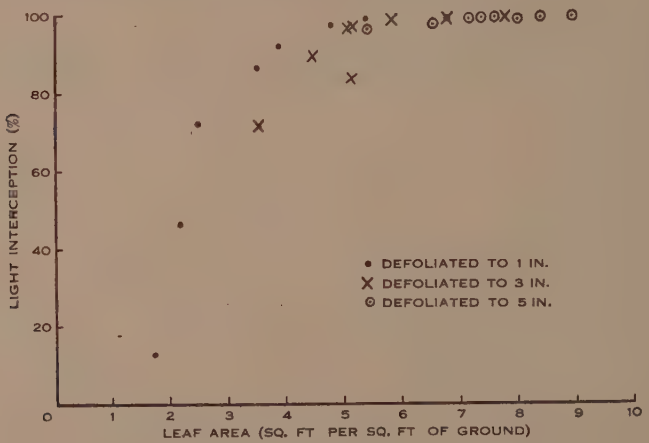

Fig. 5.—The relationship between light interception and leaf area.

The area of leaf present in the three treatments when complete light interception was first attained was approximately 5 sq. ft per sq. ft of ground (Table 1 and Fig. 5), and the herbage yield approximately 1450 lb dry matter per acre (Table 1).

Rate of growth of pasture was related to the percentage of light intercepted by the herbage, and to leaf area. Approximately constant maximum rates were maintained when sufficient foliage had been formed to intercept almost all the light (Figs. 5 and 6).

Where the initial defoliation height was 1 in. this occurred approximately 24 days after cutting (Table 1 and Fig. 2). Rate of growth before this stage of recovery increased progressively but thereafter it remained almost constant. These phases of growth are in close accordance with those recognized in a previous experiment (Brougham 1955) with a similar pasture closely defoliated at the same time of the year.

Where the pasture was cut down to 3 in. the first growth phase lasted for about 16 days but where the initial cutting height was 5 in. it lasted

only about 4 days. A slight upward trend appeared in the second phase but this was probably due to seasonal changes in temperature and light.

The more intense the defoliation the lower was the initial rate of regrowth and the longer the time taken to attain maximum growth rate; but the maximum rate attained was approximately the same under all treatments.

Changes in the growth rate of ryegrass were similar to those of total herbage yields (Table 1). For clover, the changes in growth rate were also initially the same but towards the end of the first phase of growth in all treatments the rate of growth of clover diminished markedly.

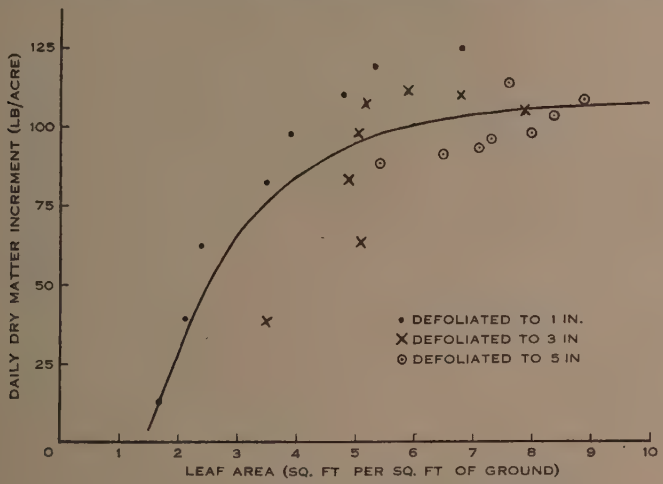

Fig. 6.—The relationship between herbage dry matter increment and leaf area (the average standard error of estimate is ± 16).

During the experimental period environmental conditions were extremely favourable for ryegrass growth and the growth rate was much faster than that of clover. The diminishing rate of growth of clover was apparently due to interspecies competition for light which became operative towards the end of the first growth phase.

Rate of increase of leaf area in each treatment followed the same pattern as rate of growth of total herbage dry matter yields (Fig. 3). Watson (1947) investigating the growth of field crops including wheat, potatoes, and sugar-beet, and Briggs, Kidd, and West (1920) from an analysis of Kreuzer's data, have established similar relationships.

The data presented in Figure 4 show that following the 3 in. and 5 in. cutting treatments maximum leaf efficiency was also approximately coincident with the attainment of complete light interception, but for pasture defoliated to 1 in. it reached a maximum before this point. Before this stage of growth, leaf efficiency increased rapidly but following this

point it gradually declined. The intensity of defoliation influenced leaf efficiency in the early stages of regrowth. The more intense the defoliation the lower the initial leaf efficiency. However, a higher maximum leaf efficiency was obtained after intense defoliation.

These changes in efficiency may be interpreted in terms of the ratio of leaf to meristematic tissue, rate of photosynthesis, rate of respiration, mutual shading, and age of leaves and chloroplasts. When plants are severely defoliated, the ratio of synthesized carbohydrate to that expended in respiration is initially low, and consequently leaf efficiency would be low when estimated by the method used in this experiment. The higher proportion of tissue such as meristem and leaf sheath (50.3 per cent.) immediately following defoliation in the 1 in. treatment compared to the 5 in. treatment (40.8 per cent.) would suggest that the initial low efficiency following cutting in the 1 in. treatment could be attributed to a higher ratio of respiration to assimilation. The decline in efficiency during the second growth phase was probably due to mutual shading of leaves although leaf age (Singh and Lal 1935) and chloroplast age (Clendinning and Gorham 1950) may also be important determinants of efficiency. The higher maximum efficiency following severe defoliation may have been due to the bulk of the leaves being at the optimum age.

The results presented above have a direct bearing on pasture management. They suggest that, for maximum production of herbage, the amount of leaf left following mowing or grazing should be sufficient to ensure complete interception of light so that pasture growth is maintained at the maximum rate. In this experiment pasture defoliated to 5 in. yielded approximately 20 per cent. more herbage than pasture defoliated to 1 in. during the experimental period. During the first 14 days of regrowth the difference in yield was about 100 per cent. However, the defoliation height associated with maximum subsequent rate of growth will vary with pasture type, weather, and season of the year, and much more detailed field work is required to determine the long-term effects of these factors on botanical composition and yield.

V. Acknowledgments

The author acknowledges the assistance of Mr. A. C. Glenday of the Applied Mathematics Laboratory, Department of Scientific and Industrial Research, Wellington, who designed the experimental layout and fitted the mathematical curves to the experimental data. The assistance of Misses D. P. Owen and D. Murphy in determining the leaf areas and botanical compositions at each sampling is also much appreciated.

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FLOWERING TIME IN SUBTERRANEAN CLOVER

By F. H. W. Morley* and C. I. Davern*

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Summary

Genotype-environment interactions have been observed in the flowering times of a number of varieties of subterranean clover (*Trifolium subterraneum* L.) grown in different locations. These interactions are interpreted in terms of the effects of photoperiod and temperature on flowering. Sensitivity to photoperiod may be independent of sensitivity to temperature. Flowering times of some strains, although simultaneous in one environment, may differ in another environment. This is due to variation in the relative importance of different components of the environment in controlling flowering time. These strain differences in flowering-time physiology are shown to be related to the climate of the strain's natural habitat. The importance of a knowledge of flowering physiology, and the recognition of the existence of genotype-environment interactions, are discussed in relation to plant breeding and introduction.

I. Introduction

Trifolium subterraneum L. is a long-day plant (Aitken 1955) in which the node and time of initiation of the first flower primordium vary considerably between strains. The time of flowering of a particular variety has been shown by Aitken, and the work reported here, to be affected by the photoperiod and temperature of the growing period.

Long-day plants generally do not initiate flower primordia if the length of day is less than a particular value. This value shows strain variation. In addition, some strains will not flower unless they have been exposed for some period to temperatures below a certain level. This minimal requirement may also vary between genotypes (Lang 1952). The low-temperature requirement may be satisfied by appropriate exposure of the developing embryo (Gregory and Purvis 1938; Aitken 1955) of germinating seed (vernalization) or of the vegetative plant.

The photoperiod requirement decreases with decreasing night temperature (Roberts and Struckmeyer 1938, 1939; Lang and Melchers 1943). Aitken (1955) found that the early variety Dwalganup, if vernalized for 8 weeks, would initiate flower primordia in the dark. Further, this variety would initiate flower primordia at temperatures greater than 70°F if the photoperiod was at least 18 hr. Similar behaviour was reported by Fife and Price (1953) in sugar-beets. On the other hand, Aitken (1955) found that the late variety Tallarook remained vegetative even at photoperiods of 18-24 hr, unless it had been exposed to temperatures below 55-60°F for at least 4 weeks.

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Although temperature and photoperiod have effects which are supplementary, their relative importances have been found by Owen, Carsner, and Stout (1940) to vary among strains of sugar-beets. Thus one strain may have a critical photoperiod above average, but a cold requirement below average, and vice versa. In *Lolium* species, exposure to cold induces a "competence to flower" (Cooper 1954) and the inductive photoperiod can stimulate floral initiation only in "competent" meristems.

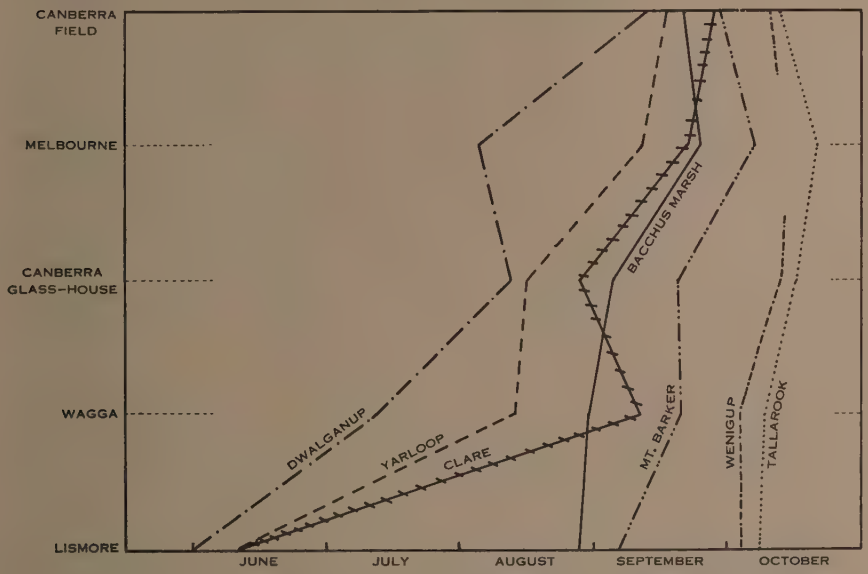

Fig. 1.—Flowering times in different environments.

No evidence for subterranean clover as yet shows such a clear-cut separation of the physiological roles of low temperature and long photoperiod. Aitken (1955) suggested that cold requirement largely determined the flowering time of different strains. However, she predicted that strains may exist in which photoperiodic sensitivity could be important.

Several observations and experiments which demonstrate the existence of large genotype-environment interactions are described. Some, which involve change in order of flowering, show that varieties which have a similar flowering time need not necessarily have a similar mechanism for inhibition (or stimulation) of flowering. It is also shown that late varieties need not have a large cold requirement for flower initiation. Finally, some results from strains collected in Morocco strongly suggest that the requirements for floral initiation can be related to the climate of the region to which the variety is indigenous.

II. Field Observations

Flowering times of seven autumn-sown varieties, in five different environments, are presented in Figure 1.

The most striking feature of these results is the existence of genotype-environment interactions. Two types of interaction are clearly discernible. The larger interaction is that between the two groups: Tallarook, Mt. Barker, Bacchus Marsh, and Wenigup, on the one hand, which show very little change in flowering date from one environment to the next, and

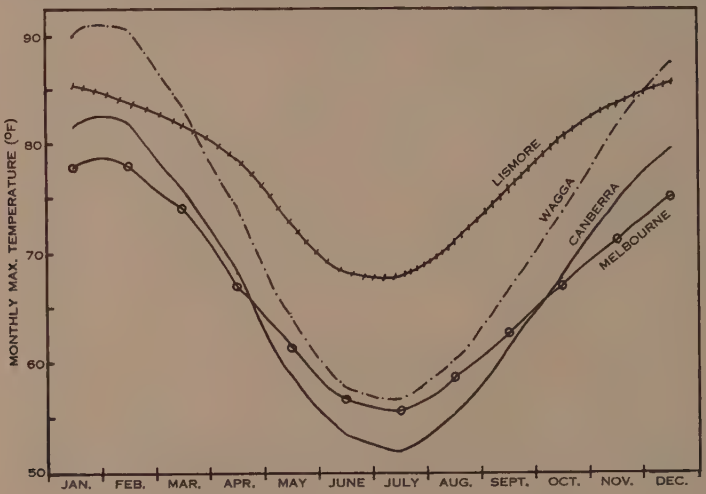

Fig. 2.—Mean monthly maximum temperatures.

Clare, Yarloop, and Dwalganup on the other hand, which have flowering dates varying widely in different environments. The smaller interaction is that between Clare and Yarloop. Clare flowered later than Yarloop in all environments except Lismore (N.S.W.) where they flowered at the same time.

The average maximum and minimum mean monthly temperatures have been plotted for four of the five environments in Figures 2 and 3, in order to indicate the temperature differences between the environments. The temperatures in the *unheated* glass-house at Canberra would be much warmer during the day, and slightly warmer at night, than the field temperatures.

The larger interaction can be explained in terms of the experimental results presented in Section III.

III. Investigation of Cold Requirement for Flowering

(*a*) *Material and Method*

The varieties Mt. Barker, Bacchus Marsh, Wenigup, Clare, Yarloop, and Northam First Early (N_1E) were subjected to various vernalization

treatments by placing germinated seeds on saturated "Wettex" pads in petri dishes after ½-weekly intervals so that they remained from ½-3 weeks in a domestic refrigerator at 5-7°C. The varieties Dwalganup and Tallarook have been adequately characterized by Aitken, and therefore were not included in this experiment. Two days before the vernalized seeds were removed from the refrigerator, seeds of each variety were sown on the "Wettex" pads in petri dishes and germinated at room temperature (control). All seedlings were transplanted and placed

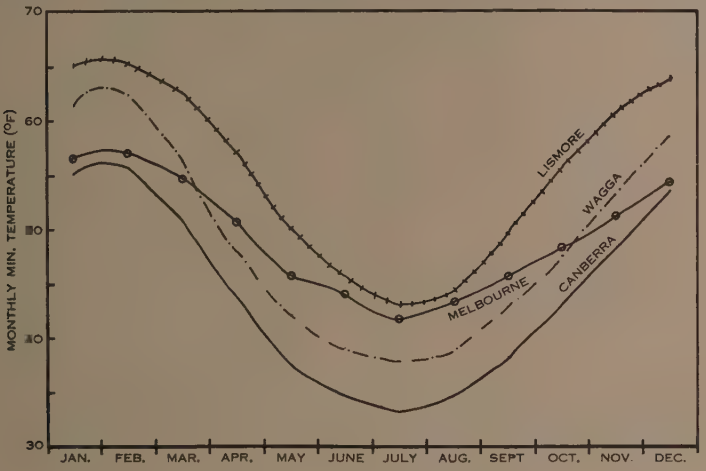

Fig. 3.—Mean monthly minimum temperatures.

individually in pots on February 14, 1955. There were six plants for each variety-treatment group. The plants were placed in the glass-house at Canberra and grown under a photoperiod of 16 hr (natural daylight supplemented by fluorescent lighting) from February 25, 1955, so that photoperiod was certainly ample for floral initiation. The experiment was terminated on May 24, 1955.

The dates and nodes of the first flower were recorded for the various treatments. The results are shown in Table 1 and Figure 4. The node of first flower was counted from the cotyledonary node (but not including it) to the node, on the axis or lateral, at which the first flower appeared.

There are methodological difficulties in using node number as an index of the developmental stage at which flowering is initiated. These are caused by both genetic and environmental factors influencing the site, whether on the axis or lateral, of the first flower. However, because of the very close correlation between time of flowering and node of flowering, it is felt that the node number of the first flower is an adequate index despite site variation. The node number of the first flower varies among the replications within treatment-variety combinations, the mean

maximum range being 4.2 ± 2.2 nodes over those treatment-variety combinations in which all six of the replicates flowered. However, the variance within groups declined sharply with increasing vernalization.

TABLE 1

COLD REQUIREMENT FOR FLOWERING: MEAN TIME OF FLOWERING (DAYS AFTER MARCH 1)
The number of plants which flowered out of the six planted for each variety is shown in brackets

Vernalization Treatment (weeks)	Varieties					
	N_1E	Clare	Yarloop	Wenigup	Bacchus Marsh	Mt. Barker
3	24(6)	25(6)	38(6)	41(6)	83(2)	All vegetative
2½	32(6)	28(6)	44(6)	59(5)*	74(3)	All vegetative
2	33(5)*	28(6)	47(6)	62(5)*	83(1)	All vegetative
1½	53(6)	41(6)	52(6)	65(4)	All veg.	All vegetative
1	44(6)	34(6)	45(6)	61(5)	84(1)	All vegetative
½	49(6)	46(6)	47(5)*	72(3)	All veg.	All vegetative
0 (control)	49(6)	52(6)	54(6)	86(3)	All veg.	All vegetative

* One plant died.

(*b*) *Results*

Mt. Barker remained vegetative and Bacchus Marsh was mostly vegetative over all treatments, indicating that the vernalization treatment

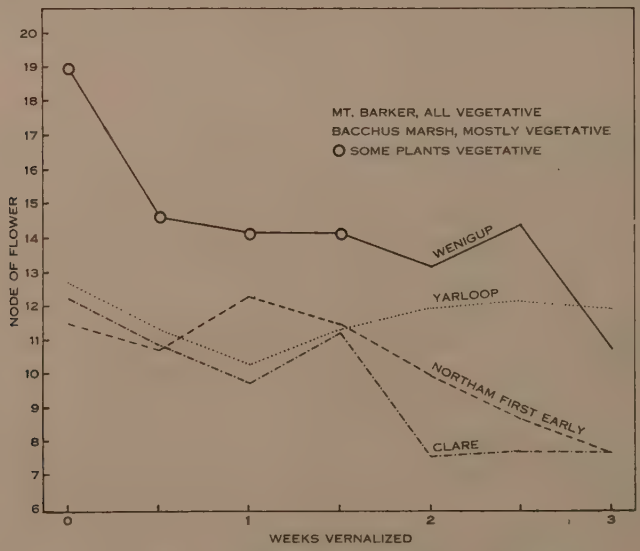

Fig. 4.—Effect of vernalization on node of flowering.

was inadequate for floral initiation. Aitken's (1955) results indicated that Tallarook required more than 3 weeks' vernalization for floral initiation

under similar conditions. Clare, N_1E, and Yarloop all flowered without vernalization. Aitken showed that Dwalganup had no cold requirement for floral initiation.

Wenigup occupies a position between the last two groups. Two weeks' vernalization were sufficient for floral initiation. Thus Wenigup, which has less cold requirement than either Bacchus Marsh or Mt. Barker, flowered later than either of them when all were sown in the autumn. However, in the present experiment, in which Wenigup flowered and Bacchus Marsh and Mt. Barker did not, the photoperiod was maintained at 16 hr. This suggests that Wenigup flowers later than Bacchus Marsh and Mt. Barker when autumn-sown because it requires a longer photoperiod.

(*c*) *Interpretation*

To clarify relationships, we introduce the equation

$$T = (n/r) + d,$$

where T = time of first flower in days from germination,

n = node number, measured from the cotyledonary node to that of the first flower,

r = mean rate, in nodes per day, of node production over the period from sowing to the initiation of the first floral node,

d = time from the initiation of the first floral node to the appearance of the first fully developed flower.

We offer the following interpretation for the larger interaction seen in Figure 1. The rate of node production (r) increased with the temperature (Aitken and Drake 1940) and was therefore larger in the warm Lismore environment than in the colder environments of Canberra, Wagga, and Melbourne. Further, d, the post-initiation phase, decreased with increasing temperature (Aitken and Drake 1940); whilst n, the node number, of varieties Clare, Yarloop, and Dwalganup, was scarcely affected by the different vernalization treatments (see Fig. 4). Thus the effect of an increase in the temperature of the growing season, on those varieties not requiring cold, was to reduce the time to flowering. However, for the cold-requiring varieties Tallarook, Mt. Barker, Bacchus Marsh, and Wenigup n was increased with an increase in the temperature of the growing season. This increase in n was apparently balanced by the increase in r and the decrease in d over the range of environments studied, the overall effect being an absence of marked differences in flowering time in different environments.

The smaller interaction, that between Clare and Yarloop, which flowered at the same time at Lismore—Clare, however, flowering later than Yarloop in the cooler locations—may be explained from the following experiment.

IV. Investigation of Flowering Reaction in Clare and Yarloop

(*a*) *Material and Methods*

Seeds of Clare and Yarloop were sown on saturated "Wettex" pads in petri dishes, and vernalized in the refrigerator for 20 days at 5-7°C. The seedlings were then transplanted and grown in the glass-house during the summer 1954-55. The number of days from planting in the glass-house to the appearance of the first mature flower was recorded.

(*b*) *Results*

The results are shown in Table 2. For comparison the number of days to flowering for Clare and Yarloop sown in autumn, at Canberra, in the field is also shown. Clare flowered before Yarloop in the summer, but later than Yarloop when sown in the autumn.

Table 2

THE EFFECT OF TIME OF SOWING ON FLOWERING TIME IN VARIETIES CLARE AND YARLOOP

Variety	Mean Days to Flower	
	Glass-house Summer-sown	Field Autumn-sown
Clare	36	88
Yarloop	48	77

(*c*) *Interpretation*

Aitken (1955) showed that Yarloop initiated flowers at the same or at a slightly lower node than Clare when sown in spring, summer, or autumn. Yet Clare flowered before Yarloop when summer-sown. This means that, in terms of the equation $T = (n/r) + d$, d (the length of post-initiation floral development) must be much shorter in Clare than Yarloop under high temperature, or r (the rate of node development) is much faster in Clare than in Yarloop under high temperatures. Either could explain the existence of the genotype-environment interaction found both in the field observations and in the Canberra experiment. Unfortunately the node of flowering and the rate of node production were not recorded for the summer sowings.

Genotype-environment interactions similar to those just described for subterranean clover have been reported. Cooper (1952) found that *Lolium rigidum* Gaud. and *L. perenne* L. S.24 headed simultaneously in one environment even though *L. rigidum* has a lower photoperiodic threshold than *L. perenne* S.24. Ear emergence in *L. rigidum* was delayed by the low spring temperatures. When grown in a warmer environment, *L. rigidum* headed 2 weeks earlier than *L. perenne* S.24. When both were

sown in late spring *L. rigidum* headed in the following summer, but *L. perenne* S.24 would not head until the following spring.

Garner and Allard (1930) demonstrated a genotype-environment interaction in the soybean, a short-day plant. Four varieties, summer-sown at Washington, D.C., took 95, 65, 55, and 25 days to flower respectively. However, when the photoperiod was artificially shortened, all four varieties flowered in 20-25 days. In subterranean clover, flowering times of strains diverge more in localities in which winter temperatures are higher.

V. Variance Heterogeneity between Strains in Flowering Time

The variances of flowering time for a number of strains sown as single-spaced plants in Canberra during the autumn of 1954 showed a striking and significant heterogeneity. Such heterogeneity may be related

Table 3

MEAN AND VARIANCE OF FLOWERING TIME AND COLD REQUIREMENT RATING
Three pairs of varieties with similar flowering times sown in March 1954

Varieties	Time to Flower after 1.ix.1954 (days)	Variance (days)	Cold Requirement for Flowering
Tallarook	39	6.0	++++
Wenigup	37	5.0	+
Mt. Barker	30	10.4	++++
Clare	28	4.7	—
Bacchus Marsh	23	9.7	+++
Cranmore	26	5.6	+

to the physiology of flowering-time control characteristic of the strains, and not to differences in genetic variation within strains, especially since this species is self-fertilized. Some of the strains were paired off on the basis of similarity in flowering time. The variance of flowering time and the cold requirements for each member of the pair are shown in Table 3.

For two of the pairs of varieties the larger variance is associated with the variety having a definite cold requirement for flowering. The third pair, Tallarook and Wenigup, do not show a significant or such a large difference in variance.

The existence of this heterogeneity for presumably genetically homogeneous varieties showing similar flowering times may be a reflection of their different physiological controls of flowering. Under the conditions of growth, Mt. Barker and Bacchus Marsh probably did not initiate flower primordia until cold requirements were fully satisfied. The larger variance may be due to partial satisfaction of this requirement by a variable, pre-maturation, natural vernalization of the seeds whilst on the

parent plant. Aitken demonstrated that pre-maturation vernalization may occur in subterranean clover. The amount of vernalization would vary among seeds matured at different times on the parent plants. Clare and Cranmore probably did not initiate flower primordia until the photoperiod was adequate. The variances of Wenigup and Tallarook were similar, even though these varieties differ in their cold requirement for flowering. This could be due to two causes.

(1) As Tallarook is a late variety its seed is produced in late spring and early summer. Temperatures may be too high to permit pre-maturation vernalization (Aitken 1955).

(2) Even though Tallarook has a cold requirement for flowering it may also have a long photoperiod requirement (Aitken and Drake 1940), and photoperiod may be the limiting factor.

VI. The Ecological Significance of Cold Requirement

Genetic variation among strains has been related to some environmental factors in a number of species. Thus Turesson (1930) and Goodwin (1944), working with *Solidago virgaurea* and *S. sempervirens* respectively, found a graded series of genetically distinguishable strains differing in their physiological requirements for flowering. This genetic variation was correlated with the geographic origin of strains, and the correlation was directly attributed to the selective effect of climate upon the species. Olmstead (1944) found geographical variation in the heading time of side oats grama. This variation was strongly correlated with latitude. Strain variation in the optimum photoperiod was the physiological basis of the correlation. Another example of adaptive variation in the photoperiodic control of flowering in a geographically widespread species was reported by Rogers (1950) in teosinte.

There is thus a dual system, photoperiod and vernalization, largely determining flowering time. The necessity for a dual system was investigated in the following experiment.

(*a*) *Material and Methods*

Eight strains collected by C. M. Donald, four from each of two localities in Morocco, were examined. The first locality was situated 21 km north of Rabat along the coast road at sea-level. Here the January isotherm is about 55°F. The second locality was south of Ifron in the Atlas Mountains at an elevation between 1700 and 1800 m. An average of 1 m. of snow lies on the ground for 2 months. Seed from each strain was given from 0 to 3 weeks' vernalization at 5-7°C. Vernalization treatments commenced on January 28, 1955, and germinated seeds from all treatments were planted out singly into pots on February 18, 1955. There were originally four plants for each strain-treatment combination, but some plants died. All plants were grown in the glass-house under a photo-

period of 16 hr (natural daylight supplemented by fluorescent lights). The date and node of the first flower were recorded, and the experiment was terminated on May 24, 1955. The glass-house was heated, and night temperatures rarely fell below 55°F during the experiment. Flowering dates of these strains when autumn-sown in the field at Canberra are given in Table 4.

TABLE 4

MEAN TIME OF FLOWERING (DAYS AFTER MARCH 1)

The number of plants which remained vegetative out of four planted for each strain is shown in brackets

Vernalization Treatment (weeks)	Strains							
	Montane				Coastal			
	47A	47B	48	50	42A	42B	42C	43A
0	[illegible]	[illegible]	[illegible]*	[illegible]	[illegible]*	[illegible]	[illegible]*	[illegible]
1	[illegible]	[illegible]*	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]*	[illegible]
2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]†	[illegible]	[illegible]	[illegible]
3 (control)	[illegible]	[illegible]	[illegible]*	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Flowering date in field‡	20	14	19	19	8	4	8	7

* One plant died.

† Two plants died.

‡ Days after September 1. For comparison, Dwalganup flowered on August 21, Yarloop on September 11, and Bacchus Marsh on September 21.

(*b*) *Results*

The node of first flower is plotted against the vernalization treatment for each strain in Figure 5. Time to flower is shown in Table 4.

The coastal strains all flowered without vernalization, whilst in three of the four montane strains more than 2 weeks' vernalization was required before all the plants in a treatment flowered. The fourth strain required more than 1 week of vernalization before all plants flowered. Further, those plants in the montane group which did flower did so at a higher node than those of the coastal group, and showed a larger response (as measured by reduction in the node number of first flower) to the cold treatment.

(*c*) *Discussion*

Even though the evidence is restricted, both in strains and regions, these results, when taking into account the other instances of climatic ecotypes mentioned earlier, seem to suggest the existence of similar ecotypes in subterranean clover. This suggestion is all the more convincing as the four strains from each region were morphologically distinct.

Strains with extended cold requirements would be at a clear disadvantage in warm coastal regions, in that the induction process would be hazardous. The observation that coastal strains show no cold requirement is therefore to be expected. But a strain of subterranean clover whose flower response is controlled solely by photoperiod would, in seasons of early opening rains, have its photoperiodic requirement satisfied in early autumn. Flowers would be initiated before winter, and in regions of cold winters the reproductive tissue would be exposed to frost damage.

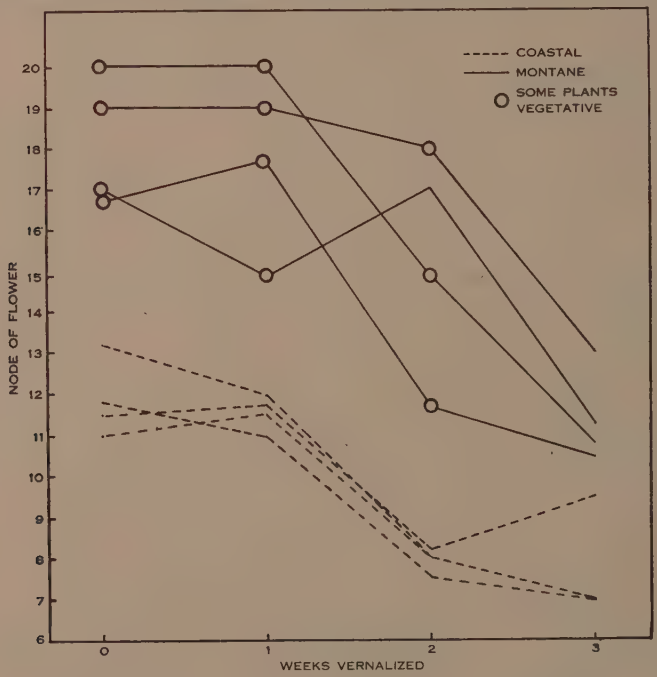

Fig. 5.—Response of Moroccan strains to vernalization.

However, a strain which had a large enough cold requirement would not initiate flowers before winter in early seasons, and thereby would escape the chance of frost damage to its reproductive tissue.

The existence of a low-temperature requirement in some strains of other species has been found to be correlated with the climate of the region from which they originated, or the temperature conditions of their growing season. Müntzing (1932) found that the winter form of *Lamium purpureum* had a cold requirement for flowering, but the summer growing form had not. Cold-requiring strains in species of wide geographical distribution were found to originate from regions of high latitude or altitude (for example, varieties of *Prunella vulgaris* and *Ranunculus acris* (Böcher 1945, 1949) and *Lolium* species (Cooper 1951, 1952, 1954)).

In these cases the existence of the cold requirement was considered as an adaptation to prevent frost damage. Such may well apply to subterranean clover.

VII. General Discussion

The precise date of first flowering in subterranean clover depends largely on the effect of temperature and photoperiod on the pre-initiation and post-initiation phases. Different genotypes may differ in their responses to these stimuli. Therefore the date of flowering at one location may be a most inexact index of that in another location. This means that if strains of certain flowering dates are to be selected, selection must be based on performance in the appropriate region.

Further, an understanding of the physiological components of systems such as flowering, and their relationship to survival in particular environments, should provide indications of the useful range of various genotypes. The relatively crude observations reported in this paper suggested to us that the varieties Clare and Yarloop should be useful at Lismore, N.S.W. This prediction had been, unknown to us, verified by field trials in that area (Crofts and Jenkins, personal communication). A precise method of testing for response to environmental stimuli, and a precise body of knowledge of both the physiological pathways leading to flowering and the genetic variation in these pathways, could lead to more efficient breeding plans and to early elimination of unpromising material.

VIII. Acknowledgments

We wish to thank Mr. K. Morgan for providing data on flowering times at Wagga Wagga, N.S.W., and Mr. H. Jenkins for providing data from Lismore, N.S.W. We are particularly grateful to Dr. L. A. T. Ballard for his patience, advice, and criticism and to Mr. J. Peak for careful observations in the field.

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STUDIES ON THE SULTANA VINE

IV. A PRUNING EXPERIMENT WITH NUMBER OF BUDS PER VINE VARIED, NUMBER OF BUDS PER CANE CONSTANT

By A. J. Antcliff,* W. J. Webster,* and P. May*

(*Manuscript received February* 27, 1956)

Summary

A pruning experiment is described in which the number of buds per vine was varied by varying the number of canes retained, the number of buds per cane being held constant. With increasing number of canes per vine, per cent. bud burst generally showed a linear decrease, and per cent. fruitful shoots, although generally not affected, showed a similar effect after years of high fruitfulness. Bunch number and weight of fresh fruit harvested increased linearly with increasing cane number. Sugar content decreased and number of mature buds produced increased with increasing cane number. Weight of fresh fruit was positively correlated with bunch number, and negatively correlated with sugar content and number of mature buds. Bunch number was positively correlated with per cent. bud burst. The yield from 10 canes in a year of low fruitfulness was no greater than the yield from 4 canes in a year of high fruitfulness. In the high year evidence suggesting overloading on vines with more than seven canes was obtained from the sugar content and bud number results. Vines returned to uniform pruning treatment after a year of high fruitfulness, the third year of the experiment, showed residual effects in the first season but not thereafter.

It is concluded that by regulating the number of canes according to the potential fruitfulness found by microscopical examination before pruning, overloading can be avoided in very fruitful seasons and the crop can be improved in years of low fruitfulness. However, complete uniformity of crop from year to year cannot be achieved.

I. Introduction

Antcliff and Webster (1955*a*) have shown that the results of a microscopical examination of buds before pruning can be used to forecast potential crop. At an early stage of the investigation the possibility of using the results as a basis for pruning recommendations to reduce the seasonal variation in potential crop presented itself. Antcliff, Webster, and May (1955) described pruning experiments in which the number of buds retained per vine was kept constant and the number and length of canes varied inversely. It was concluded that there was little hope of significantly reducing seasonal variation in potential crop in this way.

This paper reports an experiment with variation in number of buds retained per vine (pruning level). Earlier pruning experiments at the Commonwealth Research Station, Merbein (Walters 1937; Lyon and

* Commonwealth Research Station, Merbein, Vic.

Walters 1941, pp. 31-2), did not take into account seasonal variation in fruitfulness, but aimed to establish a level of pruning which could be maintained unchanged from year to year. Only three pruning levels were compared in each case. The variation among individual vines is high, and it is difficult to establish significance for the differences between particular treatments. With only three treatments it is virtually impossible to discover the trend with pruning level in the various effects studied. In the experiment where vines were pruned according to vigour each vigour class was pruned to only one pruning level, and for comparison another trial with smaller differences in pruning level was used. More complete information on these problems was therefore sought.

II. Materials and Methods

An area of sultanas of just over 1 acre at the Commonwealth Research Station, Merbein, was used. The soil was a Coomealla sandy loam (Penman *et al.* 1939) and the vines were about 20 years old. During the winter of 1950 the number of mature buds on all current season's wood was counted on all vines. The vines were then divided into classes according to the number of buds present and pruning treatments applied. Details are given in Table 1. The vines were separated into the bud number classes in the hope that variability within one class might be less than for vines taken completely at random. It was also thought that the classes would be roughly comparable with the pruning weight classes in Lyon's experiment (Lyon and Walters loc. cit.). The pruning treatments were applied to single vines, each being replicated 25 times. As far as possible all canes were pruned to 14 bud positions. On vines where this was not possible a mean cane length of 14 bud positions was approximated. The treatments were maintained unchanged from 1950-51 to 1952-53. The treatments in class A were further continued, but following a year of high fruitfulness in 1952-53 the class B vines were all pruned to eight canes in the succeeding seasons to investigate any residual effect of the previous pruning treatments. Each season per cent. bud burst, per cent. fruitful shoots, total number of bunches, weight of fresh fruit (fresh weight), sugar content of a sample of berries, and number of mature

Table 1

BUD NUMBER CLASSES, WITH PRUNING TREATMENTS APPLIED TO EACH

Class	No. of Mature Buds before Pruning	No. of Vines in Class	Pruning Treatments Applied*
A	610-790	215	3, 4, 5, 6, 7, 8
B	810-980	189	4, 5, 6, 7, 8, 9, 10

* Number of canes retained at each pruning, applied to 25 vines each, selected in randomized blocks.

buds produced were determined for each vine. To investigate further some of the effects found, dormant buds from one cane per vine in class A were microscopically examined in the winter of 1954 and 1955 to determine their per cent. fruitful buds. Also in the spring of 1955 the course of bud burst was observed daily for the class A vines according to methods described by Antcliff and Webster (1955*b*).

III. Results

(*a*) *Three Seasons of Differential Pruning Treatments in Classes A and B*

(i) *Per Cent. Bud Burst and Per Cent. Fruitful Shoots.*—Mean per cent. bud burst and per cent. fruitful shoots per vine for each pruning treatment in classes A and B from 1950-51 to 1952-53, together with the

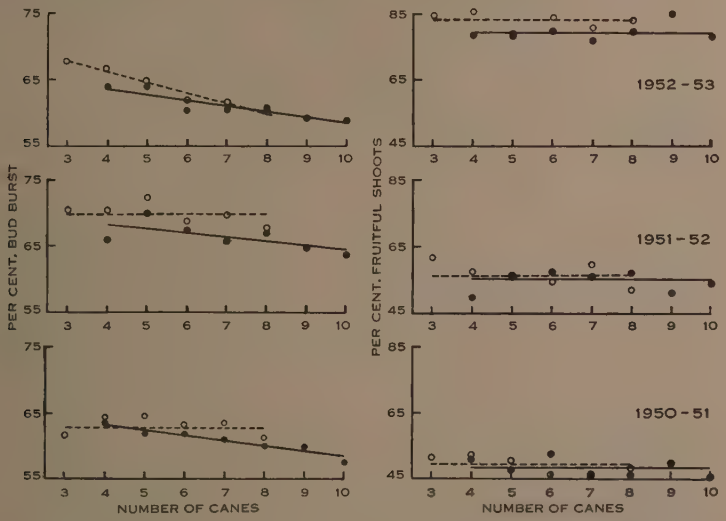

Fig. 1.—Mean per cent. bud burst and per cent. fruitful shoots per vine for each pruning treatment in each season. ○----○ Class A; ●——● class B.

regressions fitted to them, are plotted in Figure 1. In neither class did the pruning treatments affect per cent. fruitful shoots. The only differences were between seasons, and these differences were significant at the 0.1 per cent. level. The treatments did affect per cent. bud burst on at least some occasions. Where an effect was present, per cent. bud burst decreased as the number of canes retained was increased. The class B vines behaved uniformly in this respect over the three seasons. In 1951-52 per cent. bud burst was significantly higher (at the 0.1 per cent. level) than in the other two seasons, but the trend of bud burst with treatment did not differ. For the class A vines, treatment affected bud burst in only the third season. While there was some suggestion of a

decrease in per cent. bud burst with increasing number of canes retained for the five- to eight-cane treatments in the other two seasons, the means for the most severe pruning treatments did not follow this trend.

(ii) *Bunch Number and Fresh Weight.*—Mean number of bunches per vine and mean fresh weight per vine for each pruning treatment in classes A and B from 1950-51 to 1952-53, together with the regressions fitted to them, are shown in Figure 2. For both classes both bunch number and fresh weight increased with increasing number of canes retained per vine. Not only were the differences between the season means significant

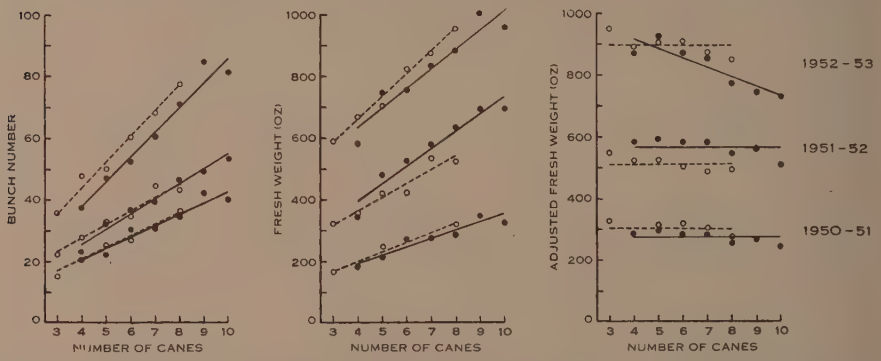

Fig. 2.—Mean bunch number per vine, mean fresh weight per vine, and mean fresh weight per vine adjusted for bunch number for each pruning treatment in each season. The adjustment is to the expected value of bunch number for seven canes in every case. ○---○ Class A; ●——● class B.

at the 0.1 per cent. level in each case, but in addition the differences in the slopes of the linear regressions for each season also reached this level of significance, the slope increasing with fruitfulness. Since per cent. fruitful shoots was not affected by pruning treatment these results are what might have been expected.

The relation between bunch number and fresh weight was examined by analysis of covariance and a very highly significant positive correlation was found. When the fresh weight means were adjusted for bunch number the upward trend with increasing cane number in every case disappeared. Indeed Figure 2 shows that there was always a downward trend in the adjusted fresh weight means with increasing number of canes per vine. In 1952-53, when the differences between the class B adjusted means reached significance, a linear regression could be fitted.

Also, as can be seen from Table 2, where the regression coefficients relating fresh weight to bunch number are given, the regression coefficient within treatments was always higher than that for the treatment means, although the difference between them was not always significant. That is, the mean fresh weight did not increase with mean bunch number from

one pruning treatment to the next as rapidly as might have been expected from the relation between fresh weight and bunch number found within treatments. Predominantly, differences in bunch number within treatments are due to differences in per cent. fruitful shoots, whereas differences in bunch number between treatment means are due to differences in the number of buds retained. It has previously been found (Antcliff and Webster 1955*a*) that fruitfulness and inflorescence primordium size are positively correlated, and it is probable that primordium size is related to the final development of the bunch. Differences in bunch number would then influence fresh weight more when caused by differences in per cent. fruitful shoots at constant pruning level than when caused by differences in pruning level at constant per cent. fruitful shoots.

TABLE 2

REGRESSION COEFFICIENTS FOR FRESH WEIGHT ON BUNCH NUMBER FOR CLASS A AND CLASS B VINES, 1950-51 TO 1952-53

Year	Regression Coefficient		Significance of Difference between Coefficients
	Between Treatment Means	Within Treatments	
Class A			
1950-51	7.7010**	9.7204***	N.S.
1951-52	9.8469***	12.6395***	*
1952-53	9.0559***	11.0015***	N.S.
Class B			
1950-51	7.5067***	9.3312***	N.S.
1951-52	11.7168***	13.9591***	*
1952-53	7.8444***	11.6490***	**

* Significant at the 5 per cent. level.
** Significant at the 1 per cent. level.
*** Significant at the 0.1 per cent. level.

As Figure 2 shows, the adjusted fresh weight values for the four-cane vines are always, and for the eight-cane vines sometimes, lower than might be expected. The vines were trained on two wires in a vertical plane. Usually the four-cane vines had one cane on each wire in each direction and the eight-cane vines two. For the other treatments, which could not be divided in this symmetrical fashion, the larger number of canes was usually on the top wire. The vines were somewhat attacked by black spot (*Elsinoe ampelina* Shear) each spring, and it is thought that shoots on the canes on the lower wire suffered more. This, if correct, would account for the greater reduction in yield on the four- and eight-cane vines, which had the highest proportion of canes on the lower wire.

Examination of Figure 2 will show that the regression line for bunch number on cane number would always cut the bunch number axis above

the origin. The constant term in the regression equation is always an appreciable positive quantity although in any particular case it is not significant. Analysis of covariance showed that bunch number was positively correlated with bud burst. When the bunch number means shown in Figure 2 are adjusted for bud burst the regression on cane number passes closer to the origin in every case. A comparison of the constant terms in the regression equations using actual and adjusted values for bunch number is shown in Table 3.

TABLE 3

VALUES OF THE CONSTANT TERM IN THE REGRESSION EQUATION OF BUNCH NUMBER ON CANE NUMBER USING ACTUAL VALUES OF BUNCH NUMBER AND VALUES ADJUSTED FOR BUD BURST

Year	Class A		Class B	
	Actual	Adjusted	Actual	Adjusted
1950-51	4.71	3.43	5.50	1.75
1951-52	9.40	8.25	5.55	2.39
1952-53	12.51	3.92	5.51	−1.02

(iii) *Sugar Content.*—Mean sugar content in degrees Brix for each treatment in classes A and B for each of the three seasons is shown in Figure 3. In 1950-51 the vines in both classes were harvested concurrently within a few days. In the following seasons, in connexion with work to be described in a later paper, the class A vines were harvested first and their harvest was spread over a longer period than that of the class B vines. The higher sugar values for the class B vines in these seasons are simply due to their later harvest. In 1951-52 the class A vines were, moreover, harvested in the order of their treatments, and the increase in sugar content with increasing number of canes is again due to differences in date of harvest. These latter results are therefore not comparable and will not be considered further. There were no significant differences between treatments for the class B vines in 1950-51 or 1951-52 although the linear regression shown for 1951-52 was highly significant. The treatment means did differ significantly in 1952-53 and could be fitted by a very highly significant linear regression. Both regressions showed that sugar content increased as the number of canes was decreased. For the class A vines there were deviations from the simple linear relations. The increase in sugar content with decreasing number of canes did not continue to the most severely pruned vines. Analysis of covariance showed that sugar content was highly negatively correlated with fresh weight, and the sugar content values adjusted for fresh weight are also shown in Figure 3. In every case (other than class A in 1951-52) the adjustment removed any significant differences between treatment means. The

increasing negative slope of the regression lines in Figure 3 for the class B vines from 1950-51 to 1952-53 must therefore be related to the increasing positive slope of the corresponding regression lines for fresh weight (Fig. 2). Although there are no significant differences in the adjusted values for sugar content, the results for 1952-53 are rather suggestive. The adjusted values increase with increasing number of canes up to the seven-cane treatment and then fall away. In 1951-52 the increase continues to the eight-cane treatment and there is no pronounced falling away. This suggests that the vines with more than seven canes in 1952-53 may have been carrying more crop than they could mature satisfactorily.

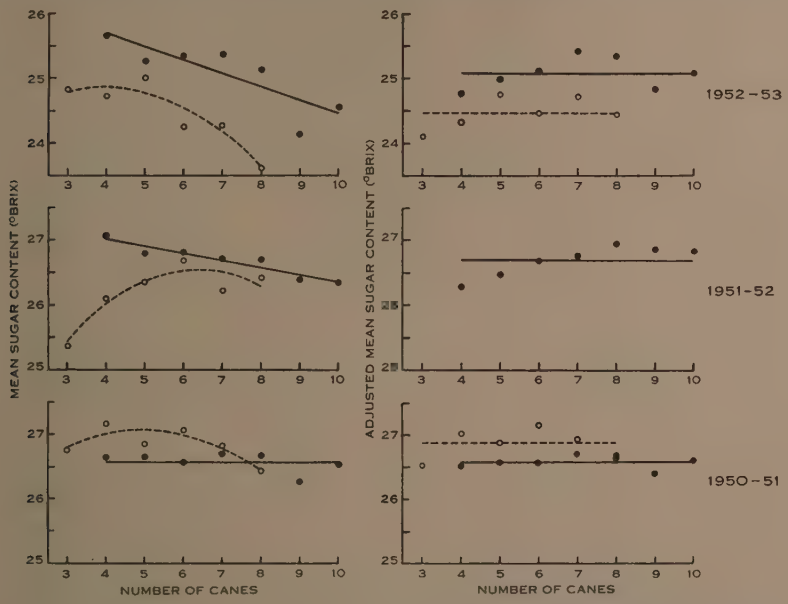

Fig. 3.—Mean sugar content in degrees Brix for each pruning treatment in each season, with values adjusted for fresh weight where applicable. ○----○ Class A; ●——● class B.

(iv) *Number of Mature Buds Produced.*—Figure 4 shows the mean number of mature buds produced per vine for each treatment in classes A and B for 1951-52 and 1952-53, when satisfactory counts were obtained. The only significant differences between treatments were for class A in 1951-52, and in this case the means could be fitted by a very highly significant linear regression. A significant linear regression could also be fitted to the class B means in 1951-52, but a quadratic is shown as it improves the fit appreciably, though not significantly, and it agrees with the trend of the adjusted means described below. Analysis of covariance showed that, except for the class A vines in 1952-53, there was always

a highly significant negative correlation within treatments between the number of mature buds per vine and the fresh weight of fruit per vine. When the treatment means were corrected for fresh weight there were highly significant differences between the adjusted means for the class B vines in both years, and for the class A vines in 1951-52. The adjusted values are shown in Figure 4; the regressions fitted are all significant.

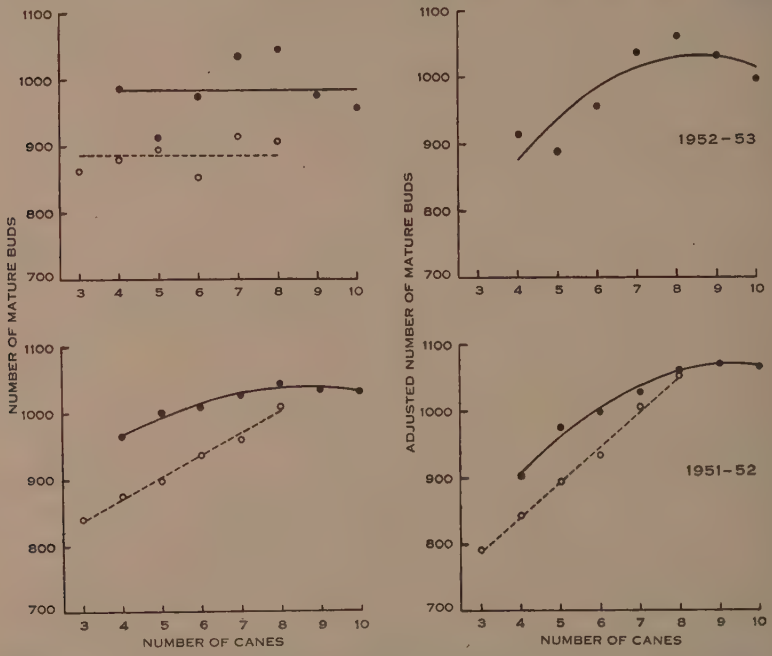

Fig. 4.—Mean number of mature buds produced per vine for each pruning treatment in 1951-52 and 1952-53, and values adjusted for fresh weight where applicable. ○----○ Class A; ●——● class B.

The adjusted means show generally similar trends to those for sugar content adjusted for fresh weight, but to a more marked degree, and similar conclusions could be drawn from them. There was a definite tendency for the bud number classes to remain distinct as is clearly indicated by the figure.

(*b*) *Results from the Class B Vines after Terminating the Pruning Treatments*

Figure 5 shows data, similar to those given previously, for the class B vines in 1953-54 and 1954-55 when all vines were pruned to eight canes. In 1953-54 there were significant differences between the formerly different pruning treatments in fresh weight and number of mature buds produced, while per cent. fruitful shoots, bunch number, and sugar content

showed corresponding trends although the differences were not significant. The trends for fresh weight and bud number were similar to those for adjusted fresh weight (Fig. 2) and adjusted bud number (Fig. 4) respectively in the previous year. By 1954-55 there were no significant differences in any respect between the groups which had previously been

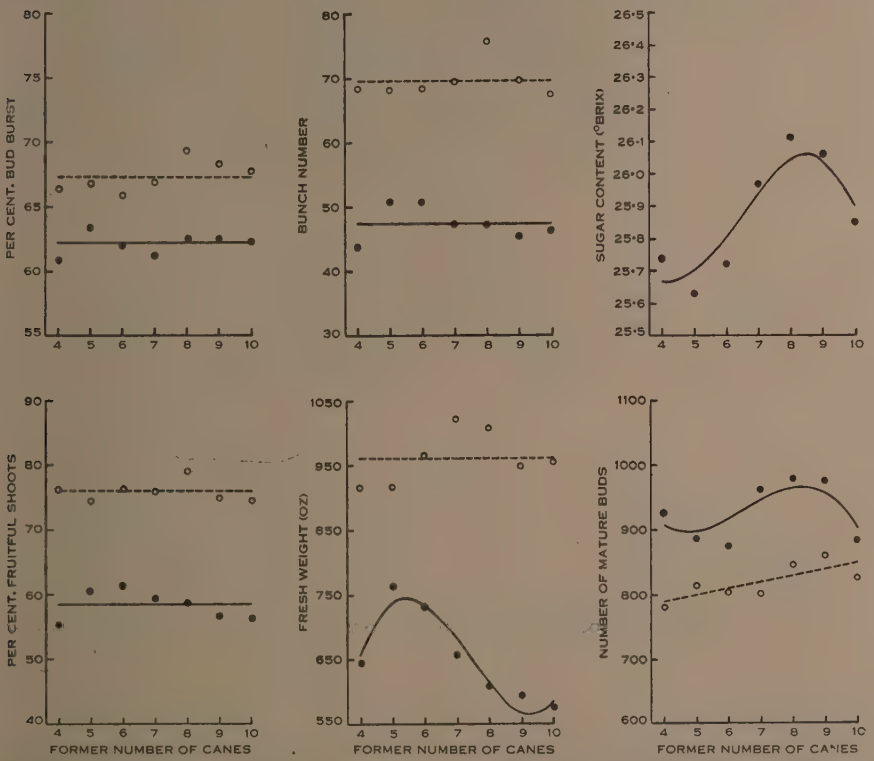

Fig. 5.—Mean per cent. bud burst, mean per cent. fruitful shoots, mean bunch number, mean fresh weight, mean sugar content, and mean number of mature buds produced per vine for the formerly differing pruning treatments in class B. There were no significant differences in sugar content but the curve shown left extremely small deviations and approached the 5 per cent. level of significance. ●——● 1953-54; ○----○ 1954-55.

pruned differently. The regression shown for number of mature buds produced was significant at the 5 per cent. level. It may indicate a slightly reduced capacity for shoot growth for otherwise equal vines where the vines had previously been pruned more severely. No sugar content determinations were made in 1954-55 so that harvesting could be expedited in an unfavourable season. There was no reason to expect significant differences.

(c) *Results from the Class A Vines in Further Seasons*

The results for the class A vines from 1953-54 to 1955-56 differed slightly in some respects from those of the first three seasons without altering their practical significance. The respects in which they differed are shown in Figure 6. This gives for each season the treatment means and regressions fitted to them for per cent. bud burst, for per cent. fruitful shoots, and for fresh weight adjusted for bunch number. There is a downward trend in bud burst with increasing number of canes retained in each season, as in 1952-53. A new feature is the downward trend in per cent. fruitful shoots with increasing number of canes, which

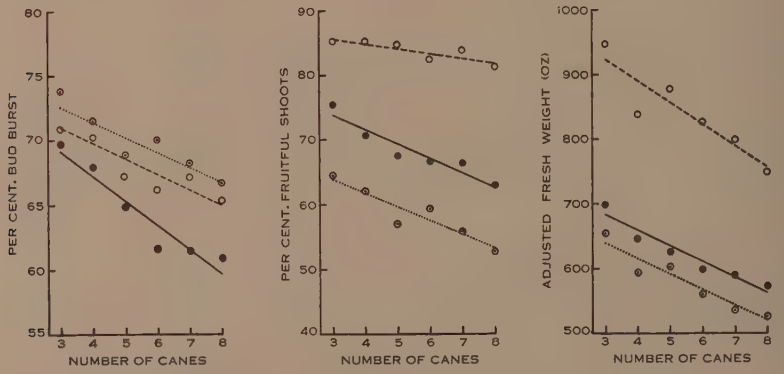

Fig. 6.—Mean per cent. bud burst, mean per cent. fruitful shoots, and mean fresh weight per vine adjusted for bunch number for each pruning treatment in class A. The adjustment is to the expected value of bunch number for seven canes in every case. ●——● 1953-54; ○----○ 1954-55; ◉ .... ◉ 1955-56.

reaches the 0.1 per cent. level of significance in each season, but is most marked in 1953-54 and 1955-56. These two seasons each follow a season of very high fruitfulness. Two factors could contribute to the decrease in per cent. fruitful shoots with increasing number of canes: a decrease in per cent. fruitful buds in dormancy, or the decrease in per cent. bud burst being predominantly due to the failure of fruitful buds to burst.

The microscopical examination of buds from one cane per vine in 1954-55 and 1955-56 failed to show any differences between treatments in per cent. fruitful buds which might be related to the differences in per cent. fruitful shoots. This may have been due to the fact that a large enough sample of canes could not be examined. In the course of bud burst study in 1955-56 no significant differences were found in mean time of bursting per vine for the various treatments, nor was there any evidence of differences between the treatments in the trend of per cent. fruitful shoots per date. Decisive differences in these trends might have clearly

indicated variations in the degree of preferential bursting of fruitful shoots, but the absence of such decisive differences does not preclude such variations. In the absence of any definite information it is suggested that the relatively small differences between treatments in per cent. fruitful shoots in 1954-55 were due to the decrease in per cent. bud burst with increasing number of canes, this decrease being predominantly due to the failure of fruitful buds to burst. In the other two seasons this effect was reinforced by differences in per cent. fruitful buds. Differences in per cent. fruitful buds, if present, should have shown up as differences in per cent. fruitful shoots for the class B vines in 1953-54 when all were pruned alike and did not differ in per cent. bud burst. Apart from the former four-cane vines there is some suggestion that the expected differences did occur (Fig. 5).

The values of fresh weight always showed a significant downward trend with increasing cane number when adjusted for bunch number, and the regression of fresh weight on bunch number within treatments was always significantly greater (at the 0.1 per cent. level) than that for the treatment means, thus confirming the results on which the conclusions in Section III(*a*) (ii) are drawn.

IV. Discussion

The results show that bunch number and yield increase linearly with increasing number of canes over the range studied, and that changes in pruning level will alter potential crop accordingly. However, the seasonal variation in fruitfulness appears to be too great to allow a uniform crop of sufficient size to be obtained each year by regulation of pruning. Thus it would have been impossible to leave sufficient canes in 1950-51 to obtain the crop obtained from four canes in 1952-53. Years of very low fruitfulness such as 1950-51 are not very frequent (Antcliff and Webster 1955*a*) but in such years it appears to be quite impossible to obtain a good crop. Certainly there is no danger of overloading the vine. In 1950-51 the increase in crop with increasing number of canes was not accompanied by any significant decrease in sugar content. In 1951-52, an average year, 9 or 10 canes gave a good crop and did not appear to strain the vines in any way. Possibly more canes could with advantage be retained in such years, but the delay in maturation with increasing crop and the extra work required both at the pruning when the canes were rolled on and at the following pruning when they have to be cut off would have to be considered. In 1952-53 a good crop was obtained from seven canes and there was some suggestion from the sugar content and bud number results that vines with more than this number of canes were overloaded.

Previous experiments did not take into account variation in fruitfulness between seasons, but aimed to establish a level of pruning which could be maintained unchanged from year to year. When fruitfulness

cannot be determined before pruning this is, of course, the most satisfactory system. Walters (1937), for example, recommended that six canes per vine be retained each year at Woorinen because with more canes the heavier crop and consequent later maturity would mean a greater risk of unfavourable weather during drying. This would apply most strongly in years of above average fruitfulness. In years of low fruitfulness more canes could be left to compensate for the lower fruitfulness without increasing this risk but this would require that such years could be forecast.

As fruitfulness can now be determined with reasonable precision before pruning (Antcliff and Webster 1955*a*) pruning recommendations can now take variation in fruitfulness into account. The first 3 years of the experiment here described covered as wide a range of fruitfulness as has been found in the Murray Valley, and were therefore very suitable for this investigation. The results from the later years fully substantiated the results from the first three seasons. The results suggest that on vines such as those used, for which 8 14-bud canes would have been regarded as a standard pruning, the number of canes retained should range from all available in years of low fruitfulness to about 7 in years of high fruitfulness, with about 10 in an average year. Leaving extra canes in low years may result in the development of untidy long shanks on the vines, but these can be removed in high years when fewer canes are required. The disappearance of any residual effect of previous pruning treatment in the second year after their termination clearly indicates that retaining canes according to fruitfulness will have no permanent effect on the carrying capacity of the vine in later seasons.

The results do not support the conclusions drawn from the pruning according to vigour experiment described by Lyon and Walters (1941). The differences between the vines with four, six, and eight canes in both class A and class B are each year generally at least as great as those obtained by Lyon and Walters. Furthermore the two classes, which presumably correspond in some way with the vigour classes of Lyon and Walters, show no consistent differences in yield between them.

V. Acknowledgments

Acknowledgments are due to Mr. R. Birtwistle, of the Division of Mathematical Statistics, C.S.I.R.O., for suggestions in connexion with the statistical work, and to the many present and former members of the staff of the Commonwealth Research Station, Merbein, who have helped in carrying out the work.

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THE CHLORIDE STATUS OF THE IRRIGATED SULTANA VINE AND ITS RELATION TO VINE HEALTH

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Summary

The chloride content of petioles from the middle leaves of shoots (which is higher than that of corresponding leaves) gives the best estimate of the chloride status of an irrigated sultana vine from bud burst until the middle of January. After this date, foliar samples may not give a reliable indication of the vine chloride status. The physiological age of the portion sampled and the normal seasonal chloride accumulation must be considered in interpreting foliar chloride values. A sample of the annual wood taken in winter may also give a suitable estimate.

On an undrained loam which bears vines showing various degrees of salt injury, there is a close relation between petiole chloride, severity of the salt symptoms, and vine health. Vines with petiole chloride levels (dry basis) of 0.8 per cent. in early December showed slight burning of basal leaf margins, while chloride levels above 1.9 per cent. in early December or 2.5 per cent. in early January were accompanied by severe burning of the basal leaves, considerable restriction of growth, and very low fruit yields.

On two other sites healthy vines with no obvious salt injury had petiole chloride contents of 1.7 per cent. in December in one case, and 2.0 per cent. in the other. Therefore a general petiole chloride level indicative of excessive salinity could not be established. To investigate whether local variations in vine health may be attributed to salt, adjacent healthy vines should be sampled in the same way for comparison, especially on those sites where leaf symptoms are absent. The leaf symptoms, as used in this study, may be assumed indicative of excess soil salts.

On the sites examined there is no relation between total sulphur or boron content of petioles or leaves and the leaf burn symptoms described. In contrast to chloride values, both the total sulphur and boron contents of leaves are higher than those of petioles.

I. INTRODUCTION

Soil salinity is widely known as a serious threat to economic crop production, particularly where irrigation is practised. The difficulty of determining and expressing the salt status of an irrigated soil has been shown by Thomas (1939), who had previously (1934) suggested using the vine leaf to diagnose excessive soil salts. His finding (1934) of a close relation between manifest salt injury and the chloride contents of leaves and soil was based on chloride determinations of samples collected during the latter part of the growing season. More information throughout the vine growth period for vines in the field was desired.

The large spatial variation in the concentration and movement of soil salts under furrow irrigation found by Thomas (1934, 1939) requires

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a time-consuming replicated soil sampling procedure to estimate the soil salt status at any given time. Since there is a continually changing pattern of soil salt concentration due to rise of salt between irrigations it is difficult to relate the soil salt status during the growing season with vine health.

Because of the limitations of soil salt analysis, the chloride status of the vine as an index of soil salt status and vine health has been further studied:

(i) To examine the integrated effect of soil salts and salt absorption processes on the vine, particularly on the seasonal accumulation of chloride in leaves and petioles;

(ii) To note whether the absorption of sulphur, boron, or sodium influences the relation between leaf and petiole chloride and manifest salt symptoms;

(iii) To note the effect of several sites on the relation between the chloride status of the vine and salt symptoms.

Sultanas only are discussed as they are the major horticultural planting in the irrigation settlements near Mildura. Because of efficient underground tile drainage systems in these settlements the salt problem has been brought under control generally throughout the horticultural lands. However, some isolated areas on various soil types where salt injury was apparent have been examined.

These findings should be relevant to those irrigated horticultural settlements established along the Murray Valley where salt problems have arisen because efficient drainage systems have not been provided or are difficult to establish.

II. Materials and Methods

Much of this work was done on a site which has been kept undrained for investigational purposes and which bears 21-year-old sultanas showing various degrees of leaf burn due to salt injury. The soil is a Coomealla loam (Penman *et al.* 1939), a type which is widespread near Mildura.

(*a*) *Field Sampling Methods*

For the foliar analyses of individual vines 30 leaves (lamina plus petiole) or petioles were sampled. For three-vine units 10 petioles or leaves were taken from each vine to give a composite sample. Duplicate samplings from single vines indicated the reliability of the sampling procedure.

The annual growth was sampled from individual vines in winter by selecting three suitable pruning canes arising from 1-year-old wood.

The vine trunks were sampled in winter. Four holes were bored with a $\frac{5}{8}$ in. bit to a depth of about $1\frac{1}{2}$ in. and the borings composited.

(*b*) *Laboratory Methods*

The plant samples were oven-dried, and ground in a Wiley mill to pass a 1 mm sieve.

For petiole and leaf samples the chloride content was measured by the electrometric titration method of Best (1929) using a cold-water extraction. In the case of the canes and trunk, reproducible results were obtained by covering the sample with N sulphuric acid for half an hour, then diluting with cold water, and titrating within half an hour.

The foliar total sulphur content was determined by the turbidimetric method of Steinbergs (1953). The petiole sodium content was measured by the method of Piper (1947) and the foliar boron content by the method of the Association of Official Agricultural Chemists (1945).

(*c*) *Vine Health Ratings*

Vines within an area of $\frac{1}{8}$ acre on the principal site have been rated for health each winter from 1948 to 1952. This health rating was based on the number and condition of the pruning canes available and the general vigour of the vine, a "good" cane being taken as one with at least 14 mature buds. The health rating was:

G	Good	vines: 12 or more good canes available
F+	Fair+	vines: 9-11 good canes available
F	Fair	vines: 6-8 good canes available
F−	Fair−	vines: 3-5 good canes available
B	Bad	vines: 1-2 good canes available, much "dieback" on canes, weak growth
B−	Bad−	vines: no good canes available, scrubby, very weak growth

"Dieback" is the distal end or tip of the cane which did not mature during the growing season.

During leaf sampling, the following symptom ratings were given for manifest salt injury to leaves:

0	No burning
1	Slight burning of basal leaf margins—about $\frac{1}{16}$ in. round the entire margin
2	Burning of basal leaf margins—about $\frac{1}{8}$ in. round the entire margin
3	Burning of basal leaves—about $\frac{1}{4}$ in. round the entire margin
4	Severe burning of basal leaves—burning increasing to intervenous areas
5	Severe burning of basal leaves and burning of middle or end leaves or both

TABLE 1

VARIATION IN CHLORIDE CONTENT DUE TO THE PHYSIOLOGICAL AGE OF LEAVES AND PETIOLES FROM THE SULTANA VINE

Sampled at fixed positions* along the shoot from vines of different health ratings. Each sample was a composite of three similar vines. Chloride content expressed as per cent. dry weight

Vine Health:	Fair+			Fair−			Bad−		Fair+		
Part Sampled:	Leaves			Leaves			Leaves†		Petioles		
Sampling Date	Basal	Middle	End	Basal	Middle	End	Basal	Middle	Basal	Middle	End
27.ix.1948	0.36			0.88			0.89		0.48		
25.x.1948	0.60	0.34		1.79	0.87		1.98		1.05	0.62	
22.xi.1948	0.90	0.40	0.21	1.84	0.85		1.82	1.55	1.50	0.87	0.47
20.xii.1948	0.87	0.57	0.25	1.52	1.15	0.67	1.87	1.68	2.00	1.37	1.08
13.i.1949	1.14	0.75	0.67	1.84	1.40	0.93	2.32	2.16	2.08	1.68	1.20
14.ii.1949	1.20	0.99	0.88	1.72	1.44	1.24		1.94	2.38	2.11	1.48
22.iii.1949	1.19	1.17	1.15	1.78	1.55	1.40			2.41	2.22	1.71

* Basal (buds 1-5), middle (buds 7-11), and end (bud 13 to end) parts of the growing shoot.

† Insufficient end leaves available for sampling.

III. Results

(a) *Chloride Contents of Leaves and Petioles*

The data for leaves and petioles are given in Tables 1-4 and Figures 1-3.

An examination of Table 1 shows that the chloride contents of leaves and petioles sampled from predetermined positions decrease from the base to the end of the growing shoot. Since the "basal" (buds 1 to 5)

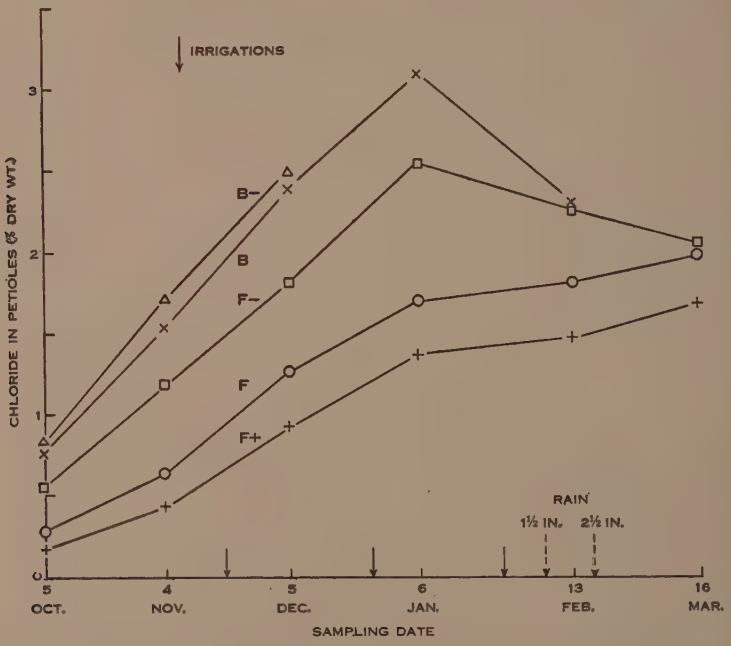

Fig. 1.—The chloride content of petioles sampled from middle leaves of the growing shoot. Each line represents the seasonal accumulation in vines of similar health rating on undrained Coomealla loam in 1949-50. Health rating: F+, fair+; F, fair; B, bad; B—, bad—. For B— vines insufficient petioles remained for sampling after December.

leaves have the highest chloride content they normally exhibit salt symptoms first. The rapid chloride accumulation in the basal leaves of weak vines causes the early death and loss of these leaves. By November 22 many basal leaves of the Bad— vines had dropped and by February 14 secondary growth was prominent. Throughout the season insufficient "end" leaves (buds 13 to end) were available for sampling from the Bad— vines which were severely salt-affected by December. Therefore "middle" leaves (buds 7 to 11) or petioles on the growing shoot are the most suitable sampling medium.

Leaves were also sampled from growing shoots arising from the basal, middle, and end portions respectively of 1-year-old canes. Results showed that for leaf sampling purposes the position of the growing shoot on 1-year-old canes is not important.

Table 1 shows, and other similar work by the author confirms, that the chloride content of leaves is much lower than that of petioles. This is probably due to the petiole's having a higher proportion of conducting tissue, as indicated by the moisture content (dry basis) of petioles being almost twice that of leaves.

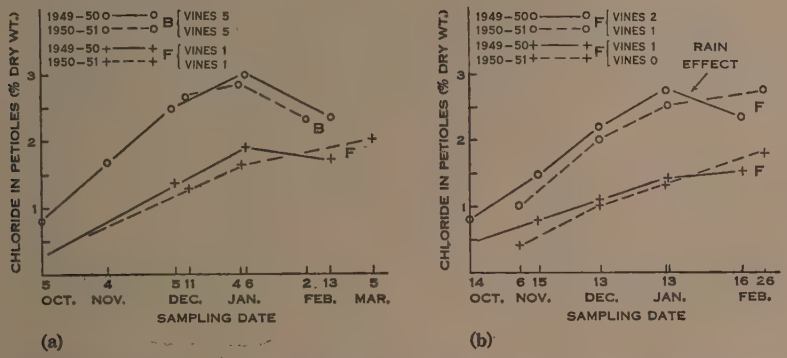

Fig. 2.—The chloride content of middle petioles from the same vines sampled in successive years. (*a*) Undrained Coomealla loam. (*b*) Undrained Nookamka sandy loam. Figures in the legend represent leaf symptom ratings in early December.

Establishment of this relation between petiole chloride and leaf chloride led to concentration of later work on petioles from middle leaves of the growing shoot.

Figures 1, 2(*a*), and 2(*b*) are representative of several sites examined as regards the seasonal accumulation of chloride in middle petioles, which is practically linear with respect to time until early January, irrespective of site. During the maximum shoot growth period (mid October to mid January) the more severely salt-affected vines accumulate chloride at a higher rate than those less affected by salt (Fig. 1).

Examinations of Figures 1, 2(*a*), and 2(*b*) show that for vines with relatively high chloride content there is a large decrease in chloride levels between January and February. To check that this decrease in 1949-50 was not due to extensive sampling throughout the growing season the same vines were sampled only in the latter part of the following growing season. The 1950-51 results for Bad vines (Fig. 2(*a*)) indicate that the decrease is not due to excessive removal of leaves but rather to the death and loss of middle leaves in December-January on vines severely salt-affected early in the season. This leaf loss occurs commonly when petiole

chloride levels reach 3.0-3.5 per cent. Figures 2(*a*) and 2(*b*) show that only after January was there appreciable difference between the two years in the trends of petiole chloride content of the less salt-affected vines. Rain caused this effect in such vines on both sites and this is discussed later. Further, in some cases the chloride accumulation rate of vines decreases after early January. This is illustrated by the Fair vines in 1950-51 (Fig. 2) when there was no rain between December and March.

TABLE 2

YEARLY VARIATION OF PETIOLE CHLORIDE VALUES AND LEAF SYMPTOM RATINGS OF SULTANA VINES

Middle petioles sampled December and January; chloride expressed as per cent. dry wt.

Sample	Month of Sampling	1949-50		1950-51		1951-52	
		Chloride	Symptoms	Chloride	Symptoms	Chloride	Symptoms
1	December	1.66	1			2.59	4
	January	2.49	2			3.17	5
2	December	1.90	1			2.36	3
	January	2.53	2			2.96	4
3	December	1.74	1	1.39	0	1.80	1
	January	2.18	1	1.86	0		
4	December			2.03	2	1.50	0

The consistency from year to year of petiole chloride levels from the same vines has been examined on several sites. Figure 2 shows that for vines on any one site showing similar leaf symptoms from year to year the petiole chloride variation is small when compared at the same period each year up to early January. Where large annual chloride variations occur in the same vines sampled at similar times, as in Table 2, the petiole chloride variation is closely followed by corresponding changes in leaf symptoms.

(*b*) *Relation Between Petiole Chloride Content and Vine Health*

Unless otherwise stated, the following data apply to undrained Coomealla loam, the principal site studied.

(i) *Health Rating.*—For both sampling dates of Table 3 there is a highly significant rise in petiole chloride with decline in the health of vines rated the following winter. This is also illustrated in Figure 1. The absence of end leaves from vines in the weaker health ratings (Table 1) indicates the pronounced inhibition of shoot growth by salt.

(ii) *Yield.*—Results in Figure 3 suggest a continuous decrease in yield (dry weight calculated from fresh weight and Baumé reading according to Lyon and Walters (1941)) with increasing petiole chloride content

in December. The Fair+ vines normally yield at the rate of 2 tons/acre, which is a good crop for this soil type.

TABLE 3

RELATION BETWEEN PETIOLE CHLORIDE CONTENT AND VINE HEALTH

Middle petioles sampled from individual vines rated in the winter of 1950 on undrained Coomealla loam. Chloride expressed as per cent. dry weight

Sampling Date	Petiole Chloride in Vines of Rating					Mean Petiole Chloride
	F+	F	F−	B	B−	
5.xii.1949	1.05 ± 0.07	1.29 ± 0.07	1.83 ± 0.06	2.48 ± 0.06	2.59 ± 0.11	1.81 ± 0.03
6.i.1950	1.51 ± 0.07	1.76 ± 0.07	2.51 ± 0.06	3.08 ± 0.06	3.34* ± 0.11	2.39 ± 0.03
Mean	1.28 ± 0.11	1.52 ± 0.11	2.17 ± 0.09	2.78 ± 0.09	2.97 ± 0.17	2.10
No. of samples	5	5	7	7	6	

* Only two vines had sufficient leaves available for sampling.

(iii) *Leaf Symptoms.*—Vines within the health rating of Fair+ had middle petiole chloride values as low as 0.8 per cent. in early December, when they first showed slight salt symptoms which intensified only slightly

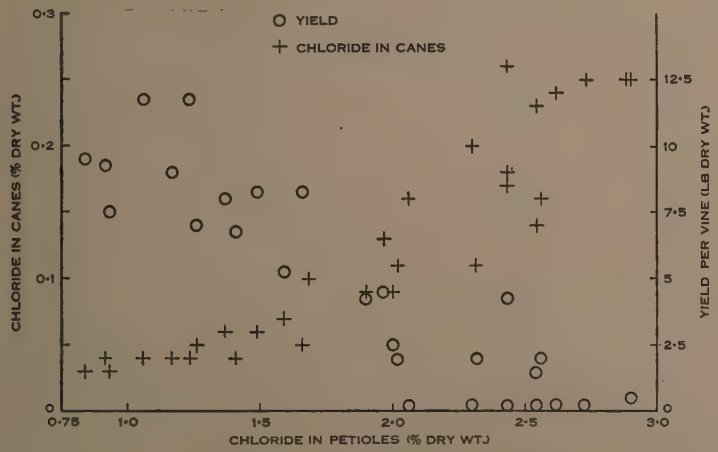

Fig. 3.—The relation between the chloride contents of middle petioles, annual wood, and yield of sultanas on undrained Coomealla loam. Petioles were sampled in early December, canes in the following winter. Yield (calculated dry weight) is given to the nearest ¼ lb. A yield of 10.2 lb/vine is equivalent to 2 tons/acre.

by February. On the other hand, the Bad vines showed the first symptoms in October and the degree of leaf burning increased rapidly with the season, as a result of which there occurred premature leaf fall and

development of secondary growth. Severe leaf burning accompanied those chloride levels associated with serious restriction of growth and yield, namely, 1.9 per cent. in early December or 2.5 per cent. in early January. The close relation between petiole chloride content and leaf symptoms is further indicated in Plate 1, which shows the increased burning of basal leaves with increasing basal petiole chloride values.

Examinations for 2 years on six other sites, some drained and others undrained, on soils ranging from Barmera sandy loam to Nookamka clay loam, indicate a fair relation between middle petiole chloride content and severity of symptoms within the site. However, from site to site the symptom rating of 1 in early December does not occur at similar chloride values. On two sites, symptoms were not evident for chloride levels of 1.7 and 2.0 per cent. respectively, while on other sites, chloride values of 1.0 to 1.3 per cent. were generally accompanied by slight salt symptoms. In no cases were severe symptoms shown for chloride values below 2.0 per cent. in early December. Figure 2(*b*) shows that some Fair vines with a symptom rating of only 2 in early December had chloride levels in December and January which, on the principal site studied, were associated with severe leaf burn and restriction of growth.

(*c*) *Composition of the Salt in Petioles and Leaves*

Penman *et al.* (1939) reported that soils in the Merbein district contain higher sulphate levels than those in other Murray Valley irrigation areas. On the principal site examined, in the top 3 ft (the vine root zone), soil chloride ranged from 18 to 40 parts per 100,000 of air-dry soil, the corresponding total soluble salts* ranged from 110 to 190, and soil pH† rose from 8.9 units in the surface 6 in. to 9.5 units in the third foot. The soil was sampled in August, 12 weeks after irrigation.

Middle petiole and leaf samples selected from vines showing slight and severe salt symptoms were analysed for total sulphur and sodium for comparison with chloride levels. The results (Table 4) show that, unlike the chloride content, total sulphur accumulation is not related to the severity of manifest salt symptoms. This was also evident for petioles examined from other soil types. From the limited number of analyses, there seems to be a close relation between chloride and sodium accumulation.

On several of the sites examined, vines exhibited symptoms of boron excess as reported by Hansen (1945). Such symptoms, quite distinct (particularly until early January) from those caused by a high chloride content, were evident for boron levels above 35 to 40 p.p.m. in the middle

* Derived from specific conductivity and expressed as equivalent NaCl parts per 100,000 air-dry soil.

† Measured by the glass electrode. Both determinations were made on a 1:5 soil-water suspension at 20°C.

petioles sampled from two sites in both February and March. Above this level the severity of boron-excess symptoms varied and was not consistent with boron values. On undrained Coomealla loam, the boron contents of petioles from Fair+, Fair−, and Bad vines, all showing moderate boron-excess symptoms in February, were 85, 52, and 44 p.p.m. respectively. The corresponding chloride levels were 1.34, 1.97, and 2.42 per cent. and leaf symptom ratings were 1, 3, and 5. Such results indicate that boron does not complicate the relation between petiole chloride and salt symptoms. The boron content of petioles is much lower than that of leaves which, on the principal site, contained above 400 p.p.m. in April.

TABLE 4

CHLORIDE, TOTAL SULPHUR, AND SODIUM CONTENTS OF MIDDLE PETIOLES AND LEAVES FROM SULTANA VINES SHOWING VARIOUS SALT SYMPTOMS ON COOMEALLA LOAM

Sample	Sampling Date	Symptoms	Cl (m-equiv./100 g dry wt.)	S	Na
Petioles					
1*	21.xii.1949	0	29	12	33
2	21.xii.1949	0	45	11	45
3	21.xii.1949	1	30	12	n.d.†
4	11.xii.1950	1	28	12	31
5	11.xii.1950	4	56	10	60
6	21.xii.1949	4	52	n.d.	71
7	21.xii.1949	5	71	n.d.	71
8	3.xii.1951	1	33	12	n.d.
9	3.xii.1951	4	59	10	n.d.
Leaves					
8	3.xii.1951	1	9	18	n.d.
9	3.xii.1951	4	27	16	n.d.

* Drained site.
† Not determined.

(*d*) *Chloride Contents of the Annual Wood and Trunk*

Figure 3 shows a relation between the chloride contents of petioles in December and of annual wood (canes) in the following winter. For cane chloride levels above 0.1 per cent. the vines had been severely salt-affected and had given very low yields in the preceding season. Cane chloride values from the same vines with similar health rating in successive years were of the same order. The distribution of chloride within the cane is given in Table 5. From a Bad vine, the dieback was analysed separately; the chloride content of the cane was 0.23 and of the dieback 0.49 per cent. These results reflect the suitability of the cane for sampling if petiole samples are not available. Petioles are preferred because they are easier to handle than canes. The high chloride values for cane tips of the weak vines (Table 5) concur with the findings of Hayward, Long, and Uhvits (1946) for peaches.

Trunks of vines were examined in winter for storage of chloride. Chloride values ranged from 0.01 per cent. (Fair+ vines) to 0.27 per cent. (Bad− vines). Although there is an increased chloride storage in the trunk of weak, severely salt-affected vines, the chloride content seemingly varies more than that of canes both within vine health ratings and from season to season. Also the trunk is not as convenient to sample as are canes.

TABLE 5

DISTRIBUTION OF CHLORIDE WITHIN THE ANNUAL WOOD
The canes from vines of different health rating were divided into basal, middle, and end portions. Chloride expressed as per cent. dry weight

Annual Wood	Cane Chloride in Vines of Rating			
	F+	F	B	B−
Basal part of cane	0.03	0.04	0.24	0.17
Middle of cane	0.04	0.05	0.25	0.21
End of cane	0.06	0.05	0.34	0.32

IV. DISCUSSION

At any particular time, an estimate of the salt status of a vine may be modified by the following variables:

(1) the part of the vine sampled;
(2) the physiological age of the portion sampled;
(3) the time of sampling during the growing season;
(4) the time of sampling in relation to irrigation and varying transpiration rates;
(5) the salt concentration in the root medium.

As the chloride content of petioles is much higher than that of corresponding leaves, and as its seasonal accumulation, being practically linear until early January, permits interpolation when different sampling dates are used, petioles from the middle of growing shoots were adopted as the sampling medium.

The large variations in chloride values, due to the physiological age of the foliar portion sampled, emphasize the importance of the sampling technique.

The seasonal accumulation of petiole chloride, which must be considered in the interpretation of the results, does not appear to be affected by irrigations (Fig. 1), nor by normal seasonal variations in evaporation. In both years examined the mean daily evaporation from a standard tank for 10 days prior to sampling did not exceed 0.3 in. For healthy, unaffected vines on different sites the average rate of chloride accumulation during

the maximum shoot growth period was 0.01 per cent. per day. For salt-affected vines the accumulation rate, although generally higher than for unaffected vines, varied from site to site. The maximum mean daily increase was 0.024 per cent. for severely salt-affected vines on undrained Coomealla loam.

The data given indicate that petiole sampling after mid January is not reliable for comparing the salt status of vines within a season and particularly from year to year. In 1950-51 there was no rain between December and March. In 1949-50 1½ in. of rain fell 8 to 5 days before sampling in February (Fig. 1), and humid conditions prevailed during the next 2 weeks. It is thought that in 1949-50 either chloride was leached or it migrated from the vine leaf. Thomas (1934) reported that the maximum chloride concentration in petioles was in the phloem and subcuticular parenchyma, and in the blades at the periphery. The decrease in petiole chloride between January and February 1950 (Figs. 1 and 2) occurred particularly in vines whose chloride content was above 2 per cent. in early January, and this rain effect explains the considerable decrease in petiole chloride of such vines in Figure 2(*b*) in 1949-50. Both Figures 1 and 2 show that the chloride accumulation rate of vines with lower chloride values in January was also affected—owing probably to reduced absorption in such weather conditions. Mes (1954) concluded that appreciable amounts of phosphorus and other elements may be leached ("recreted") from leaves by rain. Her review mentions other authors who had found considerable losses of chlorine from leaves of various mature plants when soaked in water. Heavy thunderstorms in these irrigation areas are not infrequent in January, February, or March.

On the principal site examined shoot growth is considerably restricted when petiole chloride values are above 1.9 per cent. in early December or 2.5 per cent. in early January. At these chloride levels severe leaf burning occurs and very low fruit yields are obtained. Both waterlogging, which causes poor aeration of the root zone, and the long period during which the vines have existed in a salt environment are considered to contribute to these severe depressive effects on vines with such petiole chloride levels on this site. Hayward, Long and Uhvits (1946) reported the progressive effects of saline substrates over a long period. It should be appreciated that vine health ratings may vary, within limits, according to the annual pruning and yields of the individual vine. Also, on an undrained salty soil, vine health would not be static, owing to the varying and probably deteriorating soil conditions. For these reasons vine health surveys were made each winter. The good yields from the Fair+ vines suggest that yields and vigour of some vines may not be depressed for some seasons although the vines show slight symptoms of excess salt. Further work would be needed to establish any general relation between petiole chloride and fruit yield.

Considering all sites examined, the variation between sites was too great to establish a general petiole chloride level associated with the initial leaf symptoms. Such a value varied, within the limits of this study, irrespective of soil type or drainage. This suggests that vines that are weak or affected or both should be compared with healthy, unaffected vines on each site investigated for salinity effects. Because of the ease and rapidity of petiole sampling this can be readily done. Within individual sites sampled, the known effect of efficient drainage was reflected in the decreased petiole chloride content of vines along the drains. Therefore the vine position, relative to drains, should be similar for such comparisons. A general quantitative relationship could not be established between petiole chloride content and degree of leaf symptoms because insufficient severely salted vines were available on most sites examined. It is considered that the health of those vines with petiole chloride values below 1 per cent. in early December is not affected by soil salt. On some sites slight leaf burning may be evident at this level; but it is not known whether, on such sites, this chloride level would be associated with depression of vine health over a period of years.

Within each site investigated, the salt-affected vines (particularly those with leaf symptoms rating 2 or higher) had much higher petiole chloride levels than unaffected vines. Thus the presence of the symptoms, as used in this study, may be assumed indicative of excessive chloride absorption. The sulphur results given concur with the findings of Kretschner, Toth, and Bear (1953) who reported that large variations in the substrate sulphate level had little effect on the absorption of sulphate or of other ions in the aerial parts of various plants.

The inadequacy of soil analyses has been outlined in the Introduction. As the vine salt content represents the integrated results of the factors influencing salt absorption, sampling the vine itself would be expected to give more reliable information than soil analyses. The suitability of petiole chloride analyses to illustrate the movement of soil salts with continued irrigation is indicated.

V. Acknowledgments

The author wishes to thank various laboratory assistants of the Commonwealth Research Station, Merbein, for help in the sampling and analyses. Also, grateful thanks are due to Mr. J. G. Baldwin of this Station for helpful criticism of the manuscript, to Mr. E. A. Lawton for the photographs, and to Mr. R. Birtwistle, Division of Mathematical Statistics, C.S.I.R.O., for the statistical analyses.

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SALT INJURY IN SULTANA LEAVES

Sultana basal leaves showing increasing degrees of salt injury. The leaves were sampled November 8, 1949, and the chloride content of their petioles is expressed as per cent. dry weight. The leaf symptoms are enumerated in the text. *Top left*, normal, unaffected leaf (0.81%); *top right*, slight burning leaf margin (1.35%); *bottom left*, burning leaf margin (1.55%); *bottom right*, severe burning of leaf (2.50%).

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STUDIES ON THE EFFECT OF MALACHITE GREEN ON POTATO VIRUSES X AND Y

By A. D. Thomson*

(*Manuscript received February* 9, 1956)

Summary

A study has been made of the effect of malachite green on potato viruses X and Y using Dakota Red and Aucklander Short Top potato shoot apices in tissue culture.

No plants have been freed from either virus X or Y and there has been no statistically significant effect of the dye on virus X concentration as indicated by local lesion counts on *Gomphrena globosa* L.

Malachite green either sprayed on the leaf surfaces or watered on the soil had no effect on the systemic spread of a ringspot isolate of virus X inoculated to *Nicotiana glutinosa* L.

I. Introduction

The inhibition of tobacco mosaic virus in leaf-tissue disks floated on solutions of the dye malachite green has been reported by Takahashi (1948). However, Kirkpatrick and Linder (1954) using a vacuum infiltration technique showed only a small effect of the dye on tobacco mosaic virus.

Norris (1954) has developed a virus-free line of the potato variety Green Mountain, using a potato shoot tissue culture technique with the addition of malachite green to the culture media. Only one virus X–free shoot was obtained out of the 85 treated, although by the reaction of these on *Datura stramonium* L. it was considered that in 14 cases the malachite green had reduced the virus content to a low level.

The work described in this paper is a further study of the effect of malachite green on potato viruses X and Y using Norris's technique. Two important New Zealand potato varieties, Dakota Red and Aucklander Short Top, were used. No virus X–free plants of these two varieties have been obtained from the field (Procter 1952). Aucklander Short Top is commonly infected with both viruses X and Y (Procter 1952).

The effect of malachite green was studied in potato shoot tissue cultures with the addition of the dye to the media, and in *Nicotiana glutinosa* L. growing in pots with the dye either sprayed on the plants or watered on the soil.

II. The Effect of Malachite Green in Tissue Cultures

(*a*) *Materials and Methods*

Tissue culture techniques used in this work were similar to those used by Norris (1954), with some minor modifications.

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All Aucklander Short Top tubers used to establish shoot-tip cultures were the progeny of a single plant derived from the highest yielding line of the variety grown in New Zealand (Redmond's strain). The plant was infected with viruses X (mixture of predominantly mild with some severe strain) and Y. Similarly all Dakota tubers used were derived from a single plant selection infected with a virus X strain mixture.

Stapp and Bartels (1950) have shown that the virus X concentration decreases from the potato sprout base to the apex and Prochal (1953) has shown that virus concentration in the stem-end sprouts is lower than in the apical sprouts. In the work described here, apices from stem-end shoots were used whenever possible.

Tubers were first washed under running tap water for 2 hr and then sprouted in darkness at room temperature. The apices of sprouts that developed were removed to induce lateral shoot formation. By this means up to 15 shoot apices could be excised from a single shoot. Within this group of apices some were treated with malachite green* and others were kept as controls. It was considered that the virus content would be more uniform in apices of the same age, excised at the same time from an original shoot, than in sections from a shoot-clone divided up in culture. Apices 5 mm in length were detached and surface-sterilized for 2 min with a 25 per cent. solution of commercial sodium hypochlorite with the addition of a small amount of detergent. The shoots were then washed in a series of changes of autoclaved distilled water. Apices were initially grown on White's (1943) nutrient plus 0.7 per cent. agar and pantothenic acid (Barker 1953). The culture media were autoclaved at $7\frac{1}{2}$ lb pressure for 10 min before use. After roots had formed on these cultures they were transferred to aqueous solutions of malachite green at 4 p.p.m. (Takahashi 1948) in the liquid media, either in test-tubes or on filter paper slopes. With each batch of tips, controls were kept under the same conditions in media without the dye.

Time of malachite green treatment varied from 7 to 45 days, the average being 26 days. After treatment approximately 5 mm of the apical region of the shoots was detached and placed on nutrient agar for recovery. Small plantlets normally developed after 5 weeks and were then transferred to John Innes compost in pots.

All cultures were placed in the glass-house and experiments were conducted at intervals from August 1954 to August 1955.

When recovered plantlets in pots were 1-2 in. high, three basal leaves were ground and the extracted sap inoculated to one *Datura stramonium* L. and one *N. glutinosa* or *N. tabacum* L. plant and to two or three leaves of *Gomphrena globosa* L. (Wilkinson and Blodgett 1948). Control extracts in each case were inoculated to the opposite *G. globosa* leaves. As a further check on the presence of viruses X and Y in the tubers used to establish

* The malachite green was manufactured by British Drug Houses Ltd.

cultures, they were planted in pots after removal of apices and later indexed for virus content. All proved to be infected with virus X (Dakota) or virus X + Y (Aucklander Short Top).

(*b*) *Results*

During the course of this work 479 apices of the varieties Dakota and Aucklander Short Top were detached and placed on nutrient agar. Sixty per cent. of these remained free from bacterial and fungal contamination. 124 Dakota and 30 Aucklander Short Top plantlets survived for testing on indicator plants.

In all the preliminary growth of excised apices on nutrient agar, roots formed usually after 3-7 days and some shoot growth took place. Growth in malachite green was usually slight, but controls grew vigorously. It was of interest to note that only in a few cases did tubers form in culture. However, during the course of this work stem segments cut from plants growing in the glass-house were surface-sterilized and placed on nutrient agar. In these cultures the central axillary shoot of the nodes grew out rapidly and tubers formed in the manner already described by Mes and Menge (1954). Both Dakota and Aucklander Short Top stem segments behaved in this manner.

Many apices were lost through contamination at various stages, particularly during recovery. Also, many plantlets failed to survive when transferred to soil, but the number surviving was increased by keeping plantlets in recovery tubes until about 1 in. long.

Experiments were conducted at intervals, and up to 35 tips were included in each of the experiments. The number of controls was approximately half the number treated. However, on *G. globosa,* treated tips for testing were compared against a single control, although the presence of virus in all controls was established by inoculation to *G. globosa, N. tabacum,* or *N. glutinosa.*

No Dakota plant was freed from virus X by the treatments and no Aucklander Short Top was freed from viruses X or Y (Table 1). Table 1 indicates the average number of local lesions on *G. globosa* inoculated with treated and control samples. Comparison of local lesions is only between control and treated plants from the same tuber (the same original shoot). In many experiments no control plantlets were available for comparison with treated plantlets, and the latter were inoculated to *D. stramonium, N. glutinosa,* or *N. tabacum.* All these plantlets gave virus reactions at first test (not indicated in Table 1). Aucklander Short Top samples inoculated to *N. tabacum* in each case reacted with spot-necrosis, indicating presence of viruses X and Y (Darby, Larson, and Walker 1951).

Results from local lesion tests show there was some reduction in local lesion numbers on treated plantlets in eight experiments. In other experiments results were variable and in two experiments the number of lesions

TABLE 1

POTATO VIRUSES X AND Y IN MALACHITE GREEN–TREATED PLANTLETS

Expt. No.	Variety	Treatment (days)	Number of Treated Plantlets Surviving*	Mean No. of Lesions on *G. globosa* Leaves			Virus Reaction on *N. tabacum* or *N. glutinosa*		Virus Reaction on *D. stramonium*	
				No. of Leaves Inoculated†	Treated	Control	Treated	Control	Treated	Control
1	D	7	2	4	5	8	X	X	X	X
2	D	7	5	10	9	7	X	X	X	X
3	D	22	1	2	23	7	X	X	X	X
4	D	22	3	6	17	18	X	X	X	X
5	D	41	2	6	4	6	X	X	X	X
6	D	22	1	3	4	17	X	X	X	X
7	D	7	1	3	3	16	X	X	X	X
8	D	22	1	3	5	9	X	X	X	X
9	D	41	5	15	1	1	—	—	X	X
10	D	41	2	6	1	0‡	—	—	X	X
11	D	7	2	6	2	0‡	—	—	X	X
12	D	22	1	1	1	8	X	X	—	—
13	D	45	2	6	23	41	X	X	—	—
14	D	22	3	9	Numerous lesions	Numerous lesions	X	X	—	—
15	D	28	1	2	9	19	X	X	—	—
16	AST	26	1	2	27	40	X + Y	X + Y	—	—
17	AST	28	1	2	19	11	X + Y	X + Y	—	—
18	D	37	4	—	—	—	—	—	X	X
19	AST	37	5	—	—	—	X + Y	X + Y	X	X
20	D	35	1	—	—	—	—	—	X	X

* Includes only those in which a control also survived for comparison.

† Each plantlet inoculated to an equal number of leaves.

‡ Positive at second test.

from treated plantlets exceeded that from controls. Statistical analysis of the local lesion numbers on each leaf, using a transformation $Y = \log(X + 1)$ (cf. Kleczkowski 1949), showed that the treatments had no significant effect on virus X concentration.

III. The Effect on Malachite Green on the Systemic Spread of Virus X

(*a*) *Materials and Methods*

In a further experiment a study was made of the effect of malachite green on the systemic spread of virus X in *N. glutinosa*.

Aqueous solutions of malachite green at 4, 10, and 100 p.p.m. were either sprayed (10 ml) with an atomizer on the upper and lower leaf surfaces or applied as a soil drench (10 ml) to young *N. glutinosa* plants growing in pots under glass-house conditions. For each dye concentration 12 plants were used: 6 were sprayed and 6 watered. Twelve plants were retained as controls.

Plants were either sprayed or watered with malachite green on three successive days prior to inoculation and again on two successive days after inoculation. Plants were inoculated with a severe ringspot isolate of virus X from an unknown potato.

(*b*) *Results*

After 5 days, local ringspot and chlorotic lesions developed on inoculated leaves, followed in 8 days by systemic mottle symptoms. Control and treated plants all showed the same rate of spread and intensity of virus symptoms. Plants sprayed with 100 p.p.m. were slightly stunted compared with controls.

IV. Discussion

In the work described here no plant was freed from either potato virus X or Y. Malachite green also had no statistically significant effect on virus X concentration as indicated by local lesion counts on *G. globosa*. No study has been made of the effect of the dye on virus Y concentration, but all 30 surviving Aucklander Short Top plantlets reacted on *N. tabacum*. Norris (1954) considered the malachite green had reduced the virus X content of his plantlets to a low level because of a failure to infect *D. stramonium* at first test in some cases, and a delay in systemic symptoms of 9-19 days in other cases.

In the present work virus X concentration in treated and control plantlets was such that *D. stramonium* became infected at first test in all cases.

No effect was observed on the systemic spread of virus X in *N. glutinosa* plants either sprayed or watered with the dye. Bawden (1954) has also failed to show any effect of malachite green on tobacco mosaic

virus infection in tomato plants grown in sand and watered with solutions of the dye.

Morel and Martin (1955) have shown that virus X may not normally invade the apical meristematic region of a potato shoot and that by excision of this region virus-free plants can be obtained. Norris's virus X–free shoot could have been obtained simply by the removal of this region and not due to any effect of malachite green.

The Norris technique, however, appears to offer considerable opportunity for studying the effect of virus inhibitors on systemically infected plant material. In particular the effect of proven inhibitors of virus increase (e.g. 8-azaguanine and thiouracil) could be fully investigated.

However, in preliminary experiments with thiouracil at concentrations of 10, 50, and 100 mg/l, of the 10 Aucklander Short Top and Dakota apices treated with each concentration only 6 plantlets at 10 mg/l and 1 plantlet at 50 mg/l survived (all were Aucklander Short Top except 1 Dakota at 10 mg/l). All surviving plantlets were infected with virus X (Dakota) and viruses X and Y (Aucklander Short Top).

V. Acknowledgments

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THE EFFECT OF HIGH AIR TEMPERATURE ON PREGNANCY AND BIRTH WEIGHT IN MERINO SHEEP

By N. T. M. Yeates*

(*Manuscript received November* 30, 1955)

Summary

During the winter of 1954, 21 pregnant Peppin strain Merino ewes were subjected daily, 7 hours a day, 7 days a week, to an air temperature of 105°F dry-bulb, 92°F wet-bulb, in a psychrometric room. Nine of the ewes were heated from the date mating started and the other 12 from the 38th day of pregnancy. Birth weights of the lambs of the two groups showed no significant difference from those of 10 unheated control ewes pregnant during the same period.

However, when subjected the following year, for the same period daily throughout the whole of pregnancy, to a higher temperature, namely, 112°F dry-bulb, 98°F wet-bulb, 10 experimental ewes produced lambs having a mean birth weight 1 lb 13 oz less than that of the lambs of eight unheated control ewes. The difference is highly significant statistically.

It is concluded that pregnancy in Merinos is adversely affected by extremely high atmospheric temperature; and that this could well explain the low birth weight of lambs born following a summer gestation in tropical Queensland and in certain other parts of Australia when an unusually hot summer occurs.

I. Introduction

In a previous publication (Yeates 1953) it was shown that daily exposure of Romney Marsh ewes to high temperatures in a psychrometric room during the winter months had an adverse effect on the normal course of gestation. Groups of ewes which were experimentally heated produced lambs of lower average birth weight than those of unheated controls, and in some cases the heated animals failed altogether to lamb, whereas no such failures occurred among unheated ewes.

As the majority of sheep bred in the hot inland areas of Australia are Merinos, and as they are often pregnant through the summer months, it was decided to subject pregnant ewes of this breed, also, to high temperatures in the psychrometric room. The aim was to see if heat could explain the low birth weight of autumn lambs reported by Moule (1954) and Morley (1954) for such hot regions.

A first experiment was conducted during the winter months of 1954, but as this showed an almost negligible effect of heat at the temperatures used, a further test, using more severe hot-room conditions, was undertaken during the winter of 1955. Both experiments are described.

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II. Procedure and Results

(*a*) 1954 *Trial*

Thirty-two two-tooth, Peppin strain, maiden Merino ewes of even type, all in good nutritive state, were selected and divided randomly into three groups. A control group (A) was unexposed to heat, and two experimental groups (B and C) were subjected respectively to different heat treatments. Group A contained 10 ewes, group B 10 ewes (subsequently reduced to 9), and group C 12 ewes.

Table 1

BIRTH WEIGHT OF LAMBS, 1954

Group A Control		Group B Hot Room Each Day from Day Mating Started		Group C Hot Room from 38th Day of Pregnancy	
Wt. of Lamb	Gestation Period (days)	Wt. of Lamb	Gestation Period (days)	Wt. of Lamb	Gestation Period (days)
lb oz		lb oz		lb oz	
7 3	149	8 4	149	7 0	153
7 7	151	8 4	152	7 6½	149
7 10	149	8 6	149	7 7½	150
7 12½	147	8 6	151	7 12	153
7 13	148	8 13	153	7 12½	152
8 8	150	8 14	152	8 0	149
8 11	150	8 15	152	8 0	151
8 12	150	9 9	151	8 3	152
10 1	150	*		8 7	154
10 12½	152			8 10	154
				9 2	152
				10 4	155
Mean, 8 lb 7 oz		Mean, 8 lb 11 oz		Mean, 8 lb 3 oz	

* One ewe failed to lamb.

All heat exposures were to a temperature of 105°F dry-bulb, 92°F wet-bulb, 7 hours a day, 7 days a week. However, whereas the ewes of group B all commenced treatment on the day mating was started (March 22), treatment of each individual of group C began on the 38th day after conception was believed to have occurred. A period of 38 days exceeds two oestrous cycles, so non-return to the ram in that time was considered fairly certain evidence of pregnancy.

The heating to which the experimental ewes were subjected raised their rectal temperature (2 p.m. reading) to an average of 104.5°F and their respiratory rate to an average of 197 respirations a minute. Corresponding figures for the unheated controls were 102°F and 36 respirations a minute.

Except during the 7 hours' daily heat exposure all the ewes were run together in an open-sided pen, with a Merino ram. The ram's brisket was smeared daily with grease paint to indicate services. The sheep were all fed together, twice a day, in communal troughs. Feeding was liberal and all ewes gained in weight, additional to the weight of the products of conception, during the course of the experiment. The weight gains of the three groups were similar. Water was available at all times but that in the hot room was provided in an open vessel and its temperature tended to approximate that of the room.

TABLE 2

BIRTH WEIGHT OF LAMBS, 1955

Control		Hot Room	
Wt. of Lamb	Gestation Period (days)	Wt. of Lamb	Gestation Period (days)
lb oz		lb oz	
8 3	150	5 14	149
8 3*	153	6 8	—
8 5	152	6 13	151
9 3	151	6 14	151
9 5	151	6 14	149
9 8	153	7 13	150
10 7	149	7 14	150
10 8	150	8 0	152
		8 3	—
		8 12	151
Mean, 9 lb 3 oz†		Mean, 7 lb 6 oz†	

* This includes a 29 per cent. addition to compensate for the fact that it is a twin (Hammond 1932). As the co-twin was found dead and had been trampled it was not weighed. The membranes had not been cleared from its mouth and nostrils.

† Difference of means = 1 lb 13 oz; $P < 0.001$.

The 10 ewes of group A conceived to first service. In group B six ewes conceived to first service. One returned to the ram for six consecutive oestrous cycles and was withdrawn from the experiment. On slaughter it proved to have a constricted vagina. Of the remaining three ewes in the group, one settled to pregnancy at its second cycle; one returned for service after an irregular interval of 29 days and then conceived; the remaining ewe was served on three separate occasions, the third after an interval of 64 days, and it failed to produce a lamb. Of the 12 ewes in group C, 11 conceived to first service; the 12th conceived at its second consecutive oestrous cycle.

The individual birth weights of the lambs, all of which were singles, are presented in Table 1. Analysis of variance shows there to be no significant difference in the group means.

(*b*) 1955 *Trial*

The second experiment was conducted in an essentially similar fashion to the previous year's trial, the sheep, however, being 1 year older. The allocation of individuals to groups was again at random, but on this occasion there was only one experimental group containing 10 ewes with a control group of eight ewes for comparison. Exposure to heat was again for 7 hours a day, 7 days a week, but the temperature of the room was at the higher level of 112°F dry-bulb and 98°F wet-bulb, and on this occasion the ewes commenced heat treatment on the day following service.

The room conditions during this trial raised the rectal temperature of the ewes to an average of 106.0°F and the respiratory rate to an average of 226 respirations a minute, the corresponding figures for the unheated controls being 103.1°F and 48 respirations a minute.

All ewes of each group lambed and the individual birth weights of the lambs are listed in Table 2.

The eight control ewes all conceived to first service. Eight of the 10 experimental ewes conceived to first service. The dates of lambing of the other two ewes do not correspond with the recorded service dates, but suggest that each ewe received an additional service, later than that recorded. However, final conception was not unduly delayed in the case of either ewe.

III. Discussion

The 1954 trial showed that exposure of pregnant Merino ewes to an atmosphere of 105°F dry-bulb and 92°F wet-bulb, 7 hours a day, 7 days a week during the winter months was without effect on the birth weight of the lambs. The only suggestion of any harmful effect on pregnancy of this degree of heating lay in the return to oestrus at irregular intervals of two of nine ewes exposed to heat daily from the time mating commenced.

The 1955 trial, in which more severe heating was used, resulted in a lowering of the mean birth weight of lambs by 1 lb 13 oz.

Comparing the results of the two years' trials with the previous experiments at this Laboratory in which Romney Marsh ewes were tested, it seems clear that pregnancy is much less disturbed by heat in the Merino than in the Romney. However, if heating is sufficiently severe, even Merino ewes will show lowered birth weight of lambs, following exposure to such heat daily throughout pregnancy.

This finding, made under conditions in which heat was separated from other summer environmental variables and from nutritional effects, supports the suggestion of Moule (1954) that high atmospheric temperature during gestation might be the cause of the depressed birth weight of autumn-born Merino lambs in north Queensland. The area including

Winton, Richmond, and Kynuna, and extending towards Cloncurry and Urandangie, experiences six consecutive months, commencing in October, when mean monthly maximum screen temperatures exceed 95°F. For three of these months the mean maximum temperature is over 100°F. Temperatures of 110-115°F are common and the nights are hot. Similarly, this finding could also account for the low birth weight of spring-mated Merino ewes, following the unusually hot summer of 1951-52 in the Trangie district, N.S.W., reported by Morley (1954).

The experiments described here were conducted during winter. This procedure had the advantage of separating heat from other summer environmental factors and also of providing maximum contrast between the temperature of experimental and control ewes. However, it had the disadvantage that the effect of the 7 hours' daily heating of the experimental animals was probably largely offset by the lengthy period in cool air each night. Because less respite from heat would be available at night, pregnancy under natural summer conditions might be more seriously affected than is indicated by this experiment.

Following the almost negative result of the 1954 trial it was thought that summer length of day, rather than summer heat, might possibly be responsible for the low birth weight of lambs following gestation in summer. However, this was investigated with clearly negative results in a photoperiodic experiment which is reported separately (Yeates 1956).

IV. Acknowledgments

These experiments were conducted at the Physiology Department, University of Queensland, and the author is grateful for the hospitality and facilities accorded him. Particular gratitude is expressed to Professor W. V. Macfarlane, head of the Department, for his cooperation and helpful advice.

The experimental animals were a gift to the Physiology Department through Mr. G. Simpson from "Welltown" Merino stud, Goondiwindi, Qld., and were expertly cared for by Mr. R. C. Arentz. Mr. K. P. Haydock, Division of Mathematical Statistics, C.S.I.R.O., kindly made the statistical analyses.

The investigation was financed by grants from the Wool Research Trust Fund.

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THE EFFECT OF LIGHT ON THE BREEDING SEASON, GESTATION, AND BIRTH WEIGHT OF MERINO SHEEP

By N. T. M. YEATES*

(*Manuscript received February* 29, 1956)

Summary

Peppin strain Merino ewes, when running continuously with vasectomized rams, exhibit a definite seasonality in the incidence of oestrus. Although less decisively separated into seasons of quiescence and activity, the oestrous pattern resembles that of the British breeds of sheep. The pattern may be reversed by artificially reversing the seasonal trend of daylight duration. This is presumptive evidence that the natural breeding season of Merinos is regulated by seasonal fluctuations in the length of day.

Though there was no evidence of it in this experiment, some Merinos are believed to commence their breeding season before the days begin to shorten. This is not necessarily irreconcilable with photoperiodic control, and such animals may represent a variant genetic type.

Pregnant ewes, subjected during winter-time to a summer level of light, produced lambs of normal birth weight following a gestation of normal length. This finding helps to isolate summer heat as the factor responsible for the low birth weight sometimes reported of lambs produced out of season.

I. INTRODUCTION

During recent years it has been established experimentally that many of those breeds of sheep which have a clearly defined mating season owe such seasonality to a photoperiodic control. Breeds for which this response to light has been demonstrated include the Suffolk (Yeates 1949; Hart 1950), Blackface Mountain (Hafez 1952), and Karakul (Eaton and Simmons 1953). No light experiment involving Merinos appears to have been conducted, though there is suggestive experimental evidence that the breeding season of the Rambouillet, which is closely related to the Merino, is regulated photoperiodically (Sykes and Cole 1944).

The usual pattern of sexual behaviour is for sheep to commence breeding in late summer or autumn, the onset of ovarian activity being associated with a period (generally from 1 to 3 months, depending on breed) of decreasing daily duration of light. Although the Merino has a long breeding season (Kelley and Shaw 1943), it conforms to this pattern reasonably well. However, it differs in certain respects, notably in the capacity of at least some strains, in some regions or in some years, to commence breeding early in summer before the days have started to shorten (Watson and Radford 1955).

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The present investigation was undertaken primarily to study the response of Merino ewes to changes in the light environment, but also to reveal any unusual features of their seasonal reproductive pattern.

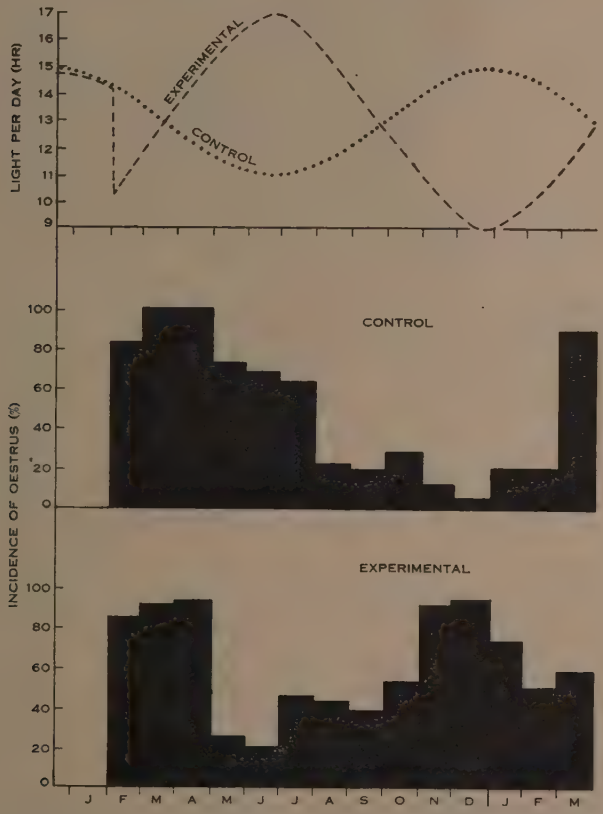

Fig. 1.—Lighting conditions to which the control and experimental groups were subjected, together with the monthly incidence of oestrus for each group. Actual oestrus is recorded as a monthly percentage of that theoretically possible, based on a 16-day cycle length.

Opportunity was also taken to observe any effect which length of daylight hours might have on the normal course of gestation and on the birth weight of lambs. For this latter experiment the same groups of ewes were mated to a fertile ram, and were maintained throughout pregnancy under the normal and the experimental light conditions.

Reversed seasonal lighting, therefore, was investigated in regard to its possible effects, firstly, on the incidence of oestrus, and secondly, on gestation and birth weight, in Merinos. Both experiments are described.

II. Procedure and Results

(*a*) *Incidence of Oestrus*

(i) *Experimental Detail.*—Sixteen two-tooth, Peppin strain, maiden Merino ewes of even type and in good nutritive state were divided randomly into a control and an experimental group of eight ewes each. The control group occupied an open-sided pen and, as no artificial lighting was used, received the daily period of lighting normal for the latitude of Brisbane (approximately 27° 30′ S.). On the other hand, by appropriate use of artificial lights and black-out arrangements, the ewes of the experimental group were subjected to conditions of reversed seasonal lighting. Changes to the duration of light of the experimental group were made weekly. The daily hours of light for each group throughout the experiment are shown in Figure 1.

Two vasectomized rams were used to detect oestrus. One remained with the light-treated group at night and with the control group in the day-time; the other, vice versa. This procedure ensured that one ram received experimental and the other normal lighting; that each ram spent a large part of each 24 hours with each group of ewes; and that ewes were exposed at all times to one or the other of the rams.

Twice daily, each group of ewes was fed a similar liberal ration, which maintained a steady and similar rise in group average body weights throughout the course of the experiments.

The experimental ewes' pen was fitted with an air extraction fan and a light-proof slot for the entry of fresh air. This arrangement prevented any overheating of the air within the pen.

The experiment started on February 4, 1954, and records of oestrus were kept until the ewes conceived following the introduction of a fertile ram in March 1955.

(ii) *Results.*—The monthly incidence of oestrus for each group of ewes may be seen in Figure 1. Breeding activity was at a high level (approaching 100 per cent.) in both groups during the first 3 months. Thereafter, the monthly trend in the incidence of oestrus was opposite in the two groups. Figure 1 and Table 1 show that mating activity of the control ewes declined to its lowest level in November and December, whereas among the ewes subjected to reversed lighting it reached its maximum during these 2 months.

(*b*) *Gestation and Birth Weight*

(i) *Experimental Detail.*—On March 4, 1955, the two vasectomized rams were withdrawn and replaced by one fertile Merino ram. This ram was run with the control ewes at night, and with the experimental group by day until lambing was due to start. The scheme of lighting used in the previous experiment was continued, so that throughout the winter

TABLE 1

MONTHLY INCIDENCE OF OESTRUS FOR INDIVIDUAL EWES OF THE CONTROL AND EXPERIMENTAL GROUPS

Each plus sign represents oestrus

Group	Ewe No.	1954										1955			Total
		F	M	A	M	J	J	A	S	O	N	D	J	F	M*	
Control	10	+	++	++	+	++	++	+	+	+			+	+	+	16
	27	++	++	+	++	++	+						+		+	12
	28	+	++	++	+	++	+	+	+	++	+	+			+	16
	42	+	++	+	++	++	+								++	11
	51		++	++	+	++	++			+			+	+	+	13
	57	++	++	+	+	+	+	+	+	+	+			+	+	14
	59	+	+	++	+		+								+	7
	70	++	+	++											+	6
Experimental	11	++	++	+	+			+			++	++	++			13
	17	+	++	++			++	+	+	++	++	+	++	+	+	18
	21	++	+	++	+		++	+	+	+	+	++	++	++	+	19
	24	+	++	++			+	+	+	+	+	++	++	+	+	16
	35	+	+	++	+	+	++	++	+	+	++	++	++	+	+	20
	44	++	+	++	+				+	++	++	+			+	13
	45	+	++	++								++	+	+	++	11
	62	+	+	++				+		+	+	++	+		+	11

* First 21 days.

the control group was in normal light, while the experimental ewes received summer hours of light.

(ii) *Results.*—The eight ewes of the control group conceived to first service and lambed. Six of the eight experimental ewes conceived and lambed. The other two had become anoestrous presumably owing to the artificially increasing day length and were not served. Of the six ewes which lambed, four conceived to first service; one conceived at a second consecutive oestrous cycle; the remaining ewe returned to the ram at an unknown date which, judging by the date of lambing, was some 6 weeks subsequent to the only recorded service. The birth weights of the individual lambs of both groups are presented in Table 2.

TABLE 2

BIRTH WEIGHT OF LAMBS, 1955

Control		Light Reversed	
Wt. of Lamb	Gestation Period (days)	Wt. of Lamb	Gestation Period (days)
lb oz		lb oz	
8 3	150	7 13	146
8 3*	153	8 4	—
8 5	152	8 5	148
9 3	151	8 11	149
9 5	151	10 8*	150
9 8	153	10 10	148
10 7	149	11 3*	150
10 8	150		
Mean, 9 lb 3 oz†		Mean, 9 lb 5 oz†	

* These animals were twins. A 29 per cent. compensatory addition has been made to their actual birth weights (Hammond 1932). As the co-twin from the control group was found dead and trampled it was not weighed. The membranes had not been cleared from its mouth and nostrils.

† Difference of means (2 oz) is not significant.

III. DISCUSSION

(*a*) *Incidence of Oestrus*

The Merinos used in these experiments, when running continuously with vasectomized rams, exhibited a definite seasonality in the incidence of oestrus. The control ewes mated most frequently in autumn and least frequently in spring, so resembling, broadly, the British breeds of sheep. The experimental ewes behaved similarly to the controls during the first 3 months and then assumed an opposite trend.

As the only difference of any known consequence in the treatment of the two groups was alteration of the light environment, it must be concluded that this induced the different seasonality in the incidence of oestrus among the experimental ewes. This, then, constitutes presumptive evidence that the breeding season of Merinos is influenced by seasonal light change under natural conditions also.

Among the 8 control ewes there was none of that type, described by Watson and Radford (1955), which commences its breeding season prior to the longest day. Four control ewes were in oestrus in October, 2 in November, and 1 in December, but these were all completing, not starting, a breeding season. The one ewe (No. 28; see Table 1) with a very long season which continued into December was anoestrous during the next 2 months and oestrus recommenced in March. Of the 8 ewes, 3 started their breeding season in January, 1 in February, and 4 in March.

It is well known that individual Merino ewes vary greatly in oestrous expression. This was shown even among the eight control animals in this experiment, for in nearly 14 months, the number of oestrous periods among individuals ranged from 6 to 16. In view of such variability it seems likely that certain strains may have a specially long breeding season, starting prior to the longest day. And as types having such a diffuse oestrous pattern are apparently the most susceptible to influences other than light, such as psychological factors (Thompson and Schinckel 1952), and possibly cold (Dutt and Bush 1955), differences in the pattern from year to year might reasonably be expected.

Unlike breeds of sheep which originated at high latitudes, the Merino's limits of seasonal breeding have probably, through its subtropical origin, been permitted a very wide range under natural selection. At any rate, if strains of Merinos exist which commence their breeding season before the longest day, this, by analogy with other species, is quite reconcilable with photoperiodic control. The mink, for example, owes its particular time of breeding to a mixed pattern of decreasing and increasing light (Hammond, Jr. 1951).

(*b*) *Gestation and Birth Weight*

The duration of gestation and birth weight of the lambs were unaffected by the experimental light environment. That the birth weight was unaffected has a bearing on the problem of low weights of Merino lambs born following an Australian summer (Moule 1954; Morley 1954). The present experiment shows that summer light during pregnancy is without harmful effect on Merinos, so apparently this factor may be eliminated as a possible cause of the problem. Heat, then, appears the likely explanation and this is supported by the results, reported previously, of heat exposure experiments with both Romney Marsh and Merino ewes (Yeates 1953, 1956).

Similarly, heat probably accounts for the low birth weight of lambs and kids born following a summer gestation in light-reversal experiments, reported from England and the United States (Yeates 1949; Eaton and Simmons 1953).

IV. Acknowledgments

The author is grateful for the cooperation and helpful advice of Professor W. V. Macfarlane, head of the Department of Physiology, University of Queensland, where this investigation was conducted.

The experimental animals, which were expertly cared for by Mr. R. C. Arentz, were a gift to the Physiology Department through Mr. G. Simpson, from "Welltown" Merino stud, Goondiwindi, Qld.

The investigation was financed by grants from the Wool Research Trust Fund.

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SOME ASPECTS OF GENE DOSAGE IN *N*-TYPE SHEEP

By S. K. Stephenson*

(*Manuscript received March* 23, 1956)

Summary

The dominant and recessive mimic genes *N* and *nr* produce increased birthcoat hairiness in New Zealand Romney lambs. This hairiness has been studied in lambs of different genotypes by three different methods.

It has been found: (i) that in individual lambs there is a gradient in hairiness from the shoulder patch to the britch; (ii) increases in hairiness with increasing dosage of the *N* and *nr* genes conform to this gradient; (iii) with any single increase in *N*-gene dosage there is a differential rate of increase in hairiness on different positions. These results are explained on the hypothesis that the primary gene action is linear, but the gradient is modified by a threshold and possibly by a ceiling effect in the production of birthcoat medullation.

I. Introduction

(*a*) *General*

The inheritance of birthcoat hairiness in the New Zealand Romney has been studied by Dry of the Massey Agricultural College. He has discovered two major independent genes and these increase the coarseness and hairiness of the birthcoat and later fleece. Their effects are similar except that one gene, *N*, acts as an incomplete dominant and the other, *nr*, as a recessive. Fraser, Ross, and Wright (1954) have shown that the primary effect of the *N* and *nr* genes is to increase the diameter and consequently the medullation of those fibres formed in the primary central and primary lateral follicles.

The birthcoat hairiness can be measured by three different methods, and these are used in this work to study the phenotypes of lambs of the different *N* and *nr* genotypes. A gradient over the body in birthcoat hairiness has been described for the New Zealand Romney lamb by Galpin (1936*a*). The interaction of this gradient with genotype appeared to be of interest, and consequently the scope of this investigation has been extended to include a study of gradients.

The three methods are outlined in some detail below. Dry (1952, 1955*a*, 1955*b*) has carried out investigations on the effects of the *N* and *nr* genes on the production of birthcoat halo-hairs and his results are included for comparative purposes.

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(*b*) *Halo-hair Descriptions*

Dry has described the effect of the *N* and *nr* genes by classification of lambs on the abundance of birthcoat halo-hairs. These are long hairy fibres that project above the rest of the fleece at birth (Dry 1933, 1933-4).

TABLE 1

CLASSIFICATION OF HALO-HAIR ABUNDANCE

Class	Dry's Classification*	General Description
VII*a*	Big reduction, big reduction, continuous reduction + side	These lambs show a reduction in abundance of halo-hairs along the side of the body and anterior to the heart girth line, but are grade VII on the standard back position
VII*b*	Reduction, big reduction, continuous reduction	Here the abundance of halo-hairs on the side of the body has increased to grade VII
VII*c*	No reduction, reduction, continuous reduction	In lambs of this phenotype, the abundance of halo-hairs on the mid-dorsal line anterior to the withers has increased to grade VII
VII*d*	No reduction, reduction, separate reduction	The next increase of abundance of halo-hairs occurs on the shoulder region and separates two regions of less than grade VII abundance, one on the side of the neck, and the other, called the shoulder patch reduction, on the region just behind the armpit
VII*e*	No reduction, no reduction, reduction	Here there is grade VII abundance of halo-hairs on all body regions except the shoulder patch
VII*f*	No reduction, no reduction, no reduction	Lambs of this phenotype are completely covered by grade VII abundance of halo-hairs

* These are Dry's descriptions of the extent of the reductions in abundance of halo-hairs below grade VII on the anterior regions of the body. A "big, big, continuous" reduction is one where there is a big reduction on the mid-dorsal line anterior to the withers, and a big reduction on the side of the neck, continuous with a reduction on the shoulder patch. "No reduction, reduction, separate" describes a phenotype with no reduction in the abundance of halo-hairs on the anterior mid-dorsal line, a reduction on the side of the neck, and a separate reduction on the shoulder patch.

A grading system for comparing non-*N* (+/+ . +/+) lambs (Dry 1955*a*) has been based on the abundance of halo-hairs on the standard back position (mid-dorsal line at the level of the last rib). Grade I lambs have no halo-hairs on this position, while grade VI have the highest abundance found in normal Romneys.

The discovery of the *N* and *nr* genes made it necessary to extend this classification in order to include lambs of different *N* genotypes. Halo-hair density classifications on the back were extended to include grade VII or *N*-grade, this describing a phenotype with a dense mat of halo-hairs (*N*-grade abundance of these fibres) on the standard back position (Dry 1955*a*). Grade VII is a ceiling classification, but lambs in this group can be further divided on the extent of *coverage* of the body by a grade VII *abundance* of halo-hairs. The classification adopted is set out in Table 1, and here grade VII lambs are divided into six grades, VII*a* to VII*f*.

Plate 2 shows the increasing coverage of the body by grade VII abundance of halo-hairs.

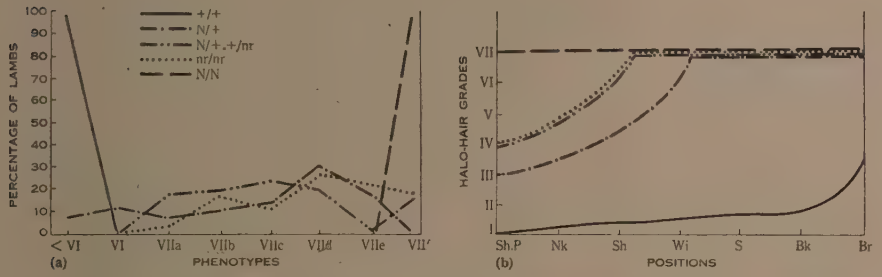

Fig. 1.—(*a*) Percentage of lambs of different phenotypes in the five genotypes. (*b*) Halo-hair gradients over the body in lambs of different genotypes.

Lambs of grades I to VI also show a variation in the abundance of halo-hairs on different regions of the body. There is a relatively high density of these fibres on the britch, poll, limbs, tail, and brisket and a low density over the front of the body anterior to the heart girth line.

The results of Dry's studies on halo-hair coverage in lambs of different genotypes are shown in Figure 1.

It is seen that the effect of increasing *N*-gene dosage is, firstly, to increase the abundance of halo-hairs on the standard back position to grade VII. Secondly, once the ceiling of grade VII is reached on this position, increasing *N*-gene dosage increases coverage of the body by a grade VII abundance of halo-hairs. This effect reaches its maximum in the *N/N* genotype. The variability of coverage between animals within a genotype, greatest in the intermediate genotypes, is discussed at length below.

(*c*) *The Fibre-type Array*

The fibres in a lock taken from the lamb's birthcoat can be divided into three distinct classes on the shape of the tip (Dry 1933, 1933-4). These three different classes of fibres are formed at different stages of

foetal and early post-natal development and Fraser, Ross, and Wright (1954) have shown that they are confined to different types of follicles.

(i) The pre-curly-tip fibres are formed in the primary central follicles and hence they are the first fibres to develop on the foetus. Typically, they consist of three distinct regions, namely, a post-natal region relatively coarse and often medullated; a pre-natal curly-tip region, finer and showing marked crimping; and a pre-natal pre-curly-tip region, usually sickle-shaped or, as in halo-hairs, long and coarsely medullated.

Fig. 2.—Fibre types. Thick lines indicate medullated portions of fibre.

(ii) Curly-tip fibres are formed in the primary lateral and early secondary follicles and they follow pre-curly-tip fibres in development on the foetus. These fibres have no pre-natal sickle-shaped tip, i.e. they consist of only a pre-natal curly-tip and post-natal regions.

(iii) Histerotrich fibres, which consist of only a post-natal region, grow in the later-formed secondary follicles. They first begin to appear above the skin about the time of birth.

These three classes of fibres have been further subdivided into fibre types on the presence or absence of medulla in the curly-tip and post-natal portions of the fibre. The different fibre types are described below. For each fibre class they are arranged in their inferred order of appearance

on the foetus, and except for checked curly-tip fibres this order corresponds to their order of decreasing medullation.

(i) *Pre-curly-tips* (*pre-CT*). *Halo-hairs* (*HH*).—Long fibres coarsely medullated throughout their length.

Halo-hair' (*HH'*).—Distinguished from halo-hairs by having a break in the medulla at the birth point.

Super-sickle A (*SSA*).—Shorter and less stout than halo-hairs and possessing a sickle-shaped tip. There is no break in the medulla at the birth point, but the pre-natal portion of the fibre below the sickle-tip is often reduced in diameter.

Super-sickle A' (*SSA'*).—Resemble super-sickle A, but have a break in the medulla at the birth point.

Super-sickle B (*SSB*).—Have a break in the medulla in the pre-natal region other than at the birth point. They may be of three types (Fig. 2).

Sickle-fibres (*Sk*).—Have no medulla in the pre-natal region below the sickle-tip, which may or may not be medullated.

Fine sickle or *super-sickle fibres*.—Have no medulla in the post-natal portion of the fibre.

(ii) *Curly-tips* (*CT*). *Hairy-tip–curly-tips* (*HTCT*).—Medullated in the pre-natal curly-tip region. Occasionally no medulla is present in the post-natal region, when they are termed checked hairy-tip–curly-tips. These fibres have been shown by Fraser, Ross, and Wright (1954) to be mainly confined to the primary lateral follicles.

Checked curly-tips.—Curly-tip fibres with the largest number of curls in the tip and thus are assumed to be the first curly-tips to develop. They are non-medullated post-natally and are followed by later formed curly-tips (fewer curls in the tip), which are coarser and often medullated.

Medullated curly-tips.—Have medulla only in the post-natal region of the fibre.

Non-medullated curly-tips.—Here this term is used exclusively for later formed curly-tips which are non-medullated post-natally.

(iii) *Histerotrichs* (*Hi*). *Medullated histerotrichs*.—Medulla present in the fibre.

Non-medullated histerotrichs.—No medulla.

Dry (1933, 1933-4) arranged the fibres in what he inferred to be their order of appearance on the foetus to form arrays. In addition to assuming that pre-curly-tip fibres appeared first and histerotrich fibres last (later verified by Fraser, Ross, and Wright (1954)), he further assumed that within the pre-curly-tip fibre group the first-formed fibres were those with the longest pre-curly-tip region and within the curly-tip fibre group the first-formed fibres were those with the greatest number of

curls in the tip. This assumption has not been proved, but it is logical and has been accepted in this paper. Fraser and Hamada (1952) and Fraser (1951) have produced evidence which suggests that the checked curly-tips are the first curly-tip fibres to develop. Dry (personal communication) has stated that in his laboratory Miss Hefferd found sweat glands attached to the follicles of curly-tips with more curls in the tip (including checked curly-tips and the bigger of the other curly-tip fibres), while the curly-tip fibres with fewer curls in the tip were in follicles without sweat glands.

Dry (1933-4) has divided fibre-type arrays into five main sections classified according to the presence or absence of different fibre types within the pre-curly-tip and early-developing curly-tip fibre classes as shown in Table 2. These main sections are called Plateau, Saddle, Ravine, Valley, and Plain. The differences, however, are not qualitative and there are intergradations.

As Plateau arrays cover a wide variation they are subdivided into Plateau P_1 (no *SSA'*, *SSB*, or *Sk*), Plateau P_2 (no *SSB* or *Sk*), and Plateau P_3 (no *Sk*). This classification is more rigid than that employed by previous workers as no arrays with sickle-fibres "in parallel" (Dry 1933-4) were classed as Plateau.

The fibre-type array classifications thus give a measure of increases in hairiness of pre-curly-tip and early-developing curly-tip fibres.

Dry's interpretation of these facts is that there is a "base" of hairiness and a "pre-natal check". The most noticeable morphological effect, attributed by Dry to the pre-natal check, is "the narrowing of the basal part of the sickle-ends" and "the fineness of the rest of the pre-natal portion of sickle-fibres". Dry (1952, and personal discussion) also considers it helpful to postulate a "base" of coarseness or medullation, or both, with the pre-natal-check reduction in medullation occurring in this base. He concludes that both check and base may vary, but the differences between arrays, as he classifies them, can be described in terms of variation in the pre-natal check, though he regards this as an oversimplification. Galpin (1936*a*) has used the terms "more depressed" and "less depressed" to describe arrays showing a greater or lesser effect of the pre-natal check. Although Dry (1933-4, 1952) regards the pre-natal check and base as forces or resulting from forces, the fibre-type array is used here purely as an anatomical classification of birthcoat hairiness. The view of the fibre-type arrays adopted here is illustrated in Figure 3, in which curves represent the several arrays in the series from Plateau to Plain. In the curve for the first array, the pre-natal check effect is the smallest and this effect reaches its greatest expression in the last curve. Figure 3*a* is based on actual fibre-type arrays. Figure 3*b* is schematic and therefore simpler.

TABLE 2

FIBRE TYPES IN DIFFERENT ARRAYS

Arrays	Fibre Types											
	HH	SSA	SSA′	SSB	Sk	Fine Pre-CT	Chkd. CT	HTCT	Med. CT	Non-med. CT	Med. Hi	Non-med. Hi
Plateau P_1	+	+						+	+	+	+	+
Plateau P_2	+	+	+					+	+	+	+	+
Plateau P_3	+	+	+	+				+	+	+		+
Saddle	+	+	+	+	+			+	+	+		+
Ravine	+		+	+	+	+		+	+	+		+
Valley			+	+	+	+	+		+	+		+
Plain						+				+		+

(*d*) *The Medullometer Test*

This was developed by Elphick (1932) and McMahon (1937). The refractive indices of benzol and the keratin of wool fibre are very similar; consequently, when wool fibres are placed in a black dish under a glass plate, and the whole irrigated with benzol, they become invisible. Hairy fibres, which contain an air-filled medulla, appear white under similar treatment. McMahon (1937) measured the reflected light by means of a photoelectric cell, this giving a quantitative measure of the degree of medullation of a wool sample.

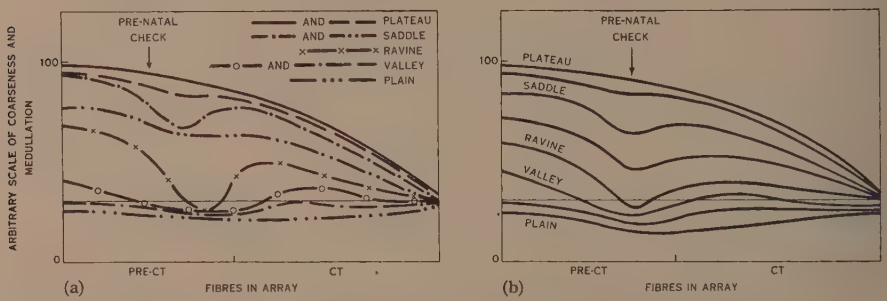

Fig. 3.—The pre-natal check effect on the coarseness and medullation of fibres in fibre-type arrays. Above the horizontal line fibres are medullated.

D. A. Ross (1950) has critically examined this method of estimating hairiness. He found that over the range used in this work, the relation between the amount of light reflected and the deflection of the indicator needle was curvilinear. The recording scale used, however, was a logarithmic one, and a test with different-sized silver coins showed that the relationship between the amount of light reflected and the reading on the scale was linear. As the amount of light reflected is a linear function of the volume of medulla in a fibre (McMahon 1937), it can be accepted that this method gives a quantitative linear estimate of increases in the volume of medulla per unit weight of wool.

Here the results obtained are used purely for comparative purposes and it is assumed that the errors of measurement (Ross 1950) are constant for the different genotypes and positions.

II. Materials and Methods

The birthcoat samples were taken from lambs obtained from two different sources. The lambs of the different *N* genotypes, born in the years 1944, 1947, 1948, and 1950, were from Dr. Dry's experimental Romney flock and the non-*N* genotype lambs, born in 1950, were from a private flock of stud Romneys.

Within a genotype, lambs for sampling were chosen at random, except for the *N*/+ genotype where there was wide variation in grade VII

coverage of halo-hairs. In the latter, lambs were divided into groups on extent of coverage and the proportion of lambs in each of these groups was calculated. Lambs for sampling were chosen at random from each group in these proportions.

The lambs were sampled in the seven standard positions, back, withers, side, shoulder patch, shoulder, neck, and britch (Plate 1, Fig. 2; also Galpin 1936*a*), except that samples from some positions of recessive-*N* and double heterozygote lambs were not available.

Table 3 shows the number of lambs, the year of birth, and the position sampled for each of the genotypes studied.

TABLE 3

DETAILS OF GENOTYPES STUDIED

Genotype	Number	Year	Positions
N/N	4	1948	All seven
	2	1950	All seven
nr/nr	4	1948	Back, withers, side, shoulder patch, shoulder, neck
	1	1947	Back, withers, side, shoulder, neck, britch
N/+.+/nr	3	1944	Back, withers, side, britch
	1	1944	Back, withers
N/+	11	1948	All seven
	1	1950	All seven
+/+	4	1950	All seven

Although uniformity in this respect would have been desirable, it was necessary to use birthcoat samples from different flocks, taken in different years.

Samples were taken at approximately 2 months after birth, as at this age fibres had not started to shed. Not all histerotrich fibres were present at this age, however, so histerotrich numbers were subject to an unknown amount of error.

The hairiness of each of these birthcoat samples was measured by both the fibre-type array and the medullometer test methods. The results obtained were used for a study of gradients in hairiness over the body and a comparison of these gradients between different genotypes.

The quantitative results (medullometer test data and percentages of fibre types) have been subjected to an analysis of variance between genotypes and positions. Where a significant overall difference has been found, the magnitude of the difference d between the individual means,

necessary for the 5 and 1 per cent. probability levels, has been calculated from the formulae

$$d = t_{0.05}\sqrt{[(2 \times \text{e.m.s.})/k_0]},$$
$$d = t_{0.01}\sqrt{[(2 \times \text{e.m.s.})/k_0]},$$

where k_0 is the harmonic mean of the number of measurements taken in each group.

III. Results

The best available objective and quantitative measure of differences in hairiness of birthcoat samples is given by the medullometer test, which measures the volume of medulla per unit weight of wool. Classification by fibre-type arrays depends on the coarseness and medullation of a few specific fibre types, namely, pre-curly-tips and, in Valley array, checked curly-tips. The two methods are thus measuring substantially the same effect, but the medullometer test results, being objective and quantitative, will be discussed first. Fibre-type arrays will then be considered, followed by a general discussion of the interrelationships of these two methods and Dry's halo-hair gradings.

(*a*) *Medullometer Test Results*

The results of the medullometer test for hairiness of the birthcoat samples are given in Table 4, which shows the means for the positions studied in each genotype. The deflection reading given by the medullometer for a standard amount of medulla was not known at the time of this work and, as the results were used only for comparative purposes, it was thought sufficient to use the deflection readings produced by a unit weight of wool. The results of the analysis of variance between genotypes and between positions are included in the table.

Table 4 shows that in all lambs there is an increase in hairiness of the birthcoat from the front of the body (shoulder patch, shoulder, neck, and withers) to the side and back and finally the britch.

The genotypes can be divided into three groups of different birthcoat hairiness as follows.

(i) The +/+ lambs show uniformly little hairiness except on the britch.

(ii) Lambs of the three genotypes *N*/+, *nr*/*nr*, and *N*/+.+/*nr* show no real differences and can be treated as a single population. This group shows little hairiness in birthcoat samples from the shoulder patch, but on all the other positions there are significant increases in hairiness above that found in +/+ lambs.

(iii) The *N*/*N* lambs show further increases in birthcoat hairiness on all positions, with the greatest increase on the shoulder patch. These increases are statistically significant except on the side, back, and britch.

TABLE 4

DIFFERENCES IN MEDULLOMETER TEST RESULTS BETWEEN POSITIONS AND GENOTYPES

Means of untransformed numbers used. *d* is the amount individual means have to differ by for difference to be significant at 5 and 1 per cent. levels

Positions	+/+	*N*/+	*N*/+.+/*nr*	*nr*/*nr*	*N*/*N*	*F* Values	*d*
Shoulder patch	16.5	64	—	65	269	$F = 27.69^{**}$ $n_1 = 3, n_2 = 21$	5% level = 65.46 1% level = 89.09
Withers	20.25	183.5	203	205	364	$F = 9.83^{**}$ $n_1 = 4, n_2 = 26$	5% level = 107.13 1% level = 144.87
Neck	17.25	192	—	173	383	$F = 13.84^{**}$ $n_1 = 3, n_2 = 22$	5% level = 111.29 1% level = 151.27
Shoulder	18.5	204	—	250	378	$F = 11.86^{**}$ $n_1 = 3, n_2 = 22$	5% level = 116.70 1% level = 158.63
Side	25.75	374	278	341	474	$F = 11.42^{**}$ $n_1 = 4, n_2 = 25$	5% level = 136.12 1% level = 184.16
Back	38.5	340	347	373	477	$F = 12.86^{**}$ $n_1 = 4, n_2 = 26$	5% level = 119.82 1% level = 161.96
Britch	116	403	453	—	516	$F = 14.99^{**}$ $n_1 = 3, n_2 = 21$	5% level = 123.70 1% level = 168.36
F values	$F = 4.83^{**}$ $n_1 = 6$ $n_2 = 21$	$F = 13.39^{**}$ $n_1 = 6$ $n_2 = 77$	$F = 9.16^{**}$ $n_1 = 3$ $n_2 = 14$	$F = 9.09^{**}$ $n_1 = 5$ $n_2 = 23$	$F = 5.89^{**}$ $n_1 = 6$ $n_2 = 35$		
d	5% level = 48.19 1% level = 65.59	5% level = 93.16 1% level = 123.71	5% level = 106.16 1% level = 147.33	5% level = 106.74 1% level = 144.81	5% level = 101.01 1% level = 135.55		

** Significant at 1% level.

Figure 4 shows the increases in birthcoat hairiness on the seven positions with increases in *N*-gene dosage.

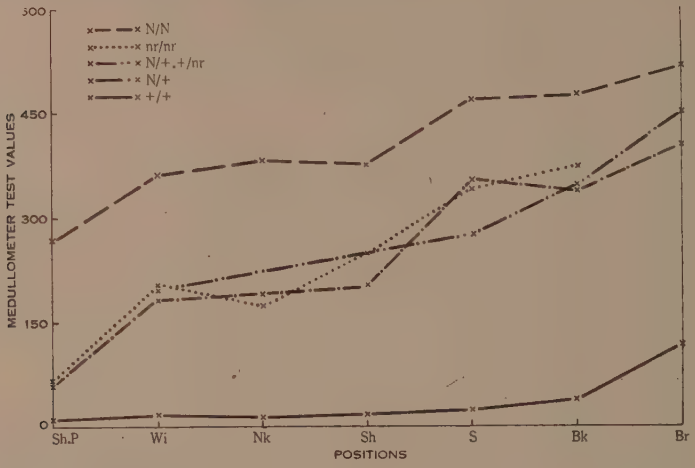

Fig. 4.—Medullometer test results. Gradients over the body in lambs of different genotypes.

(*b*) *Fibre-type Arrays*

The results of the fibre-type array work are given in Table 5, which shows the average array gradings for the positions studied in the different genotypes.

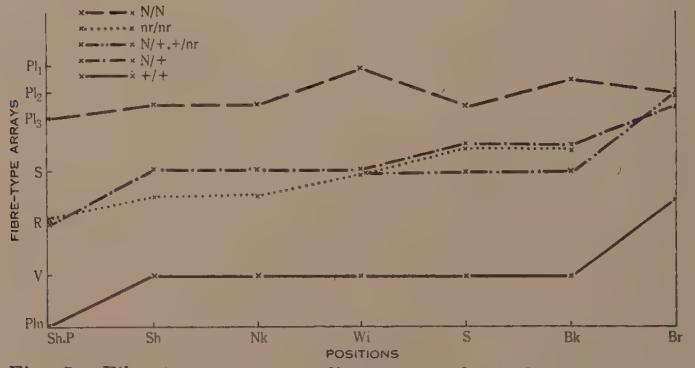

Fig. 5.—Fibre-type array gradients over the body in lambs of different genotypes.

The results are, in general, similar to those of the medullometer test work except that there appears to be a ceiling in birthcoat hairiness. Fibre-type arrays tend to this ceiling on the britch of *N*/+, *nr*/*nr*, and *N*/+.+/*nr* lambs, and on all positions, with the possible exception of the shoulder patch, of *N*/*N* lambs. This effect is shown in Figure 5.

TABLE 5

AVERAGE ARRAY GRADINGS FOR THE POSITIONS AND GENOTYPES STUDIED

Genotype	Position						
	Shoulder Patch	Shoulder	Neck	Withers	Side	Back	Britch
+/+ $n=4$	Plain	Valley	Valley	Valley	Valley	Valley	Border between Ravine and Saddle
N/+ $n=12$	Ravine	Saddle	Saddle	Saddle	Border between Plateau and Saddle	Border between Plateau and Saddle	Plateau, grade P_2 or P_3
N/+.+/*nr* $n=4$ (Bk, Wi)* $n=3$ (S, Br)†	—	—	—	Saddle	Saddle	Saddle	Plateau, grade P_2
nr/*nr* $n=5$ $n=4$ (Sh. P)‡	Ravine	Border between Saddle and Ravine	Border between Saddle and Ravine	Saddle	Border between Plateau and Saddle	Border between Plateau and Saddle	—
N/*N* $n=6$	Plateau, grade P_3	Plateau, border between grades P_2 and P_3	Plateau, border between grades P_2 and P_3	Plateau, grade P_1	Plateau, border between grades P_2 and P_3	Plateau, border between grades P_1 and P_2	Plateau, grade P_2

* Back and withers. † Side and britch. ‡ Shoulder patch.

The gradient in hairiness over the body, found using the fibre-type array method, is relatively slight in lambs of both the +/+ and *N/N* genotypes. In the *N/+*, *nr/nr*, and *N/+ . +/nr* lambs, however, there is a marked gradient which shows wide variation in different individuals. The variation is particularly marked in *N/+* lambs, where the range of phenotypes is extended to include those found in both homozygotes.

Table 6 and Figure 6 show the gradient in fibre-type arrays over the body found in different *N/+* lambs.

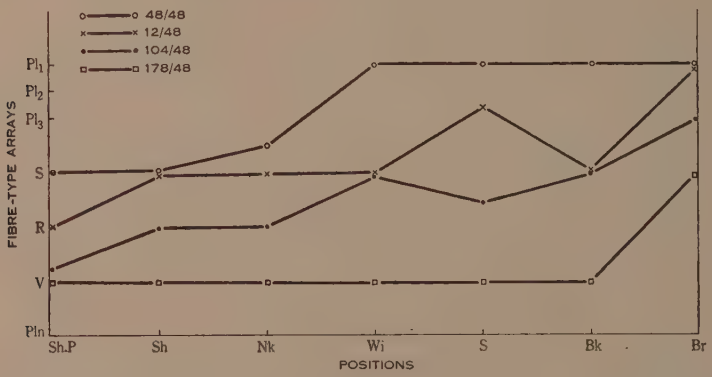

Fig. 6.—Fibre-type array gradients over the body in individual *N/+* lambs.

(*c*) *Interrelationship of Medullometer Test Results, Fibre-type Arrays, and Dry's Halo-hair Gradings*

The medullometer test results and fibre-type array studies confirm Dry's gradient in halo-hair coverage although the three methods give slightly different results. Figure 7 shows a slightly diagrammatic representation of increases in hairiness with increasing dosage of the dominant *N* gene as shown by halo-hair classifications, fibre-type array studies, and medullometer test results.

All three methods show an apparent threshold in the production of birthcoat hairiness. The ceiling shown by the fibre-type array and halo-hair studies may be a result of a ceiling to the *method* and hence a spurious effect. The ceiling to halo-hair classifications has already been mentioned, and fibre-type arrays also reach a limit grade when there is unbroken medullation in pre-curly-tip fibres. This is the situation shown by fibre-type array studies on the back and withers of *N/N* lambs, but on the side and britch there appears to be a ceiling below this limit grade, and on these positions the effect may be real. The medullometer test has no limit value in this range of hairiness, and any apparent ceiling shown by this method should be a real effect.

TABLE 6

N/+ ARRAYS

Sheep No.	Halo-hair Grading	Position						
		Shoulder Patch	Shoulder	Neck	Withers	Side	Back	Britch
16/50	III	Valley	Valley	Valley	Valley	Saddle	Ravine	Valley/Ravine
178/48	VI	Valley	Valley	Valley	Valley	Valley	Valley	Saddle
104/48	VII*a*	Ravine/Plain*	Ravine	Ravine	Saddle	Ravine/Saddle	Saddle	Plateau P_3
93/48	VII*b*	Valley	Saddle	Valley	Saddle	Plateau P_3	Plateau P_3	Plateau P_3
6/48	VII*c*	Ravine	Ravine	Saddle	Ravine/Saddle	Saddle	Plateau P_3	Plateau P_2
65/48	VII*c*	Saddle	Saddle	Plateau/Saddle	Plateau/Saddle	Plateau P_3	Saddle	Plateau P_1
12/48	VII*d*	Ravine	Saddle	Saddle	Saddle	Plateau, P_2/P_3	Saddle	Plateau P_1
41/48	VII*d*	Ravine	Saddle	Saddle	Saddle	Plateau P_3	Saddle	Plateau P_1
78/48	VII*d*	Ravine	Ravine	Saddle	Plateau/Saddle	Plateau P_3	Plateau P_2	Plateau P_1
35/48	VII*d*	Ravine	Plateau/ Saddle	Plateau P_3	Saddle	Plateau, P_2/P_3	Plateau, P_2/P_3	Plateau P_1
90/48	VII*e*	Ravine	Saddle	Saddle	Plateau P_1	Saddle	Plateau P_2	Plateau, P_2/P_3
48/48	VII*e*	Saddle	Saddle	Plateau/Saddle	Plateau P_1	Plateau P_1	Plateau P_1	Plateau P_1

* Arrays grading into those of another class, e.g. Ravine/Plain is a Ravine array bordering on a Plain.

The results from the medullometer test work, however, do not show a ceiling to hairiness; and this suggests that the medulla of a fibre can continue to increase in volume, or that fibres other than pre-curly-tips can become medullated when *N*-gene dosage is sufficient to produce continuous pre-curly-tip medulla, or possibly that both alternatives occur together.

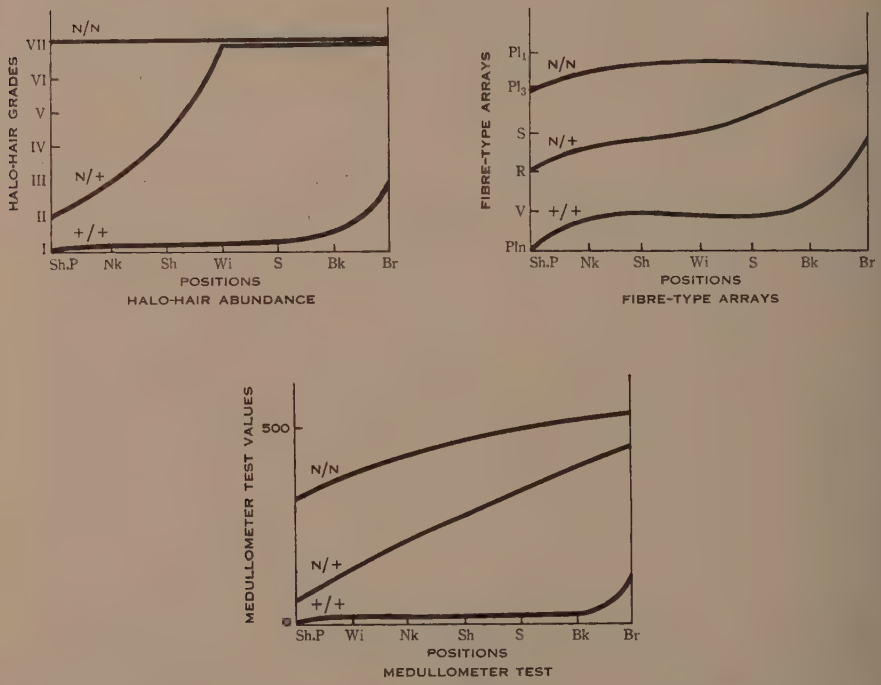

Fig. 7.—Increases in hairiness with increasing dosage of the dominant *N* gene as shown by halo-hair classifications, fibre-type arrays, and medullometer test results.

The relative increases in hairiness with increasing dosage of the dominant *N* gene, as given by the medullometer test, can be shown for the different positions by the ratio

$$(\text{increase, } N/+ \; - N/N)/(\text{increase, } +/+ - N/+).$$

On the britch, back, and side, this ratio is approximately 0.4 and on these positions the greatest increase in hairiness occurs from $+/+$ to $N/+$. On the shoulder, neck, and withers the ratio is approximately 1, and increase is linear with gene dosage. On the shoulder patch the ratio is 4.36 and here the greatest increase in hairiness occurs from $N/+$ to N/N. These ratios are plotted against positions in Figure 8.

It is seen that although there is no ceiling to hairiness as measured by the medullometer test, there is found to be a slower rate of increase in hairiness with increasing dominant *N* gene dosage on the side, back, and britch. This may possibly be the result of a limit to follicle and hence fibre size, rather than a primary genetic effect.

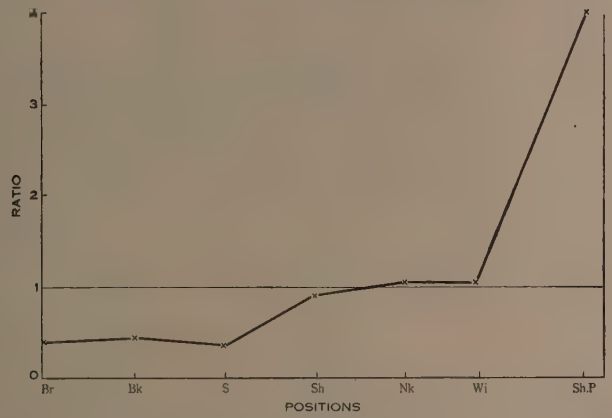

Fig. 8.—Medullometer test results. Ratio of increase $(N/+ - N/N)$ to increase $(+/+ - N/+)$.

It is possible to define three different degrees of intensity of the ceiling effect.

(i) When increase in hairiness on all positions is either linear with, or increases with, increasing *N*-gene dosage, there will continue to be a marked gradient over the body and no evidence of any approach to a ceiling.

(ii) The next stage shows a diminishing rate of increase in hairiness on the hairiest positions with increasing *N*-gene dosage, but no absolute ceiling is reached. This leads to a diminishing gradient in hairiness over the body; e.g. the medullometer test results.

(iii) Finally, when there is a definite ceiling to increases, the gradient diminishes as hairiness approaches and reaches this ceiling successively on the different positions and may finally disappear, with the result that there is a uniformly high hairiness over the whole body. This extreme result is seen with Dry's study of halo-hairs and is approached in the results of fibre-type array work.

(*d*) *Percentages of Fibres*

The percentages of different fibre types present on the different positions in different genotypes were also studied, and the results show that the distributions of the individual fibre types are closely associated with hairiness of the birthcoat.

Fine sickle-fibres and checked curly-tips reach their maximum abundance on positions and in genotypes where birthcoat hairiness is low. Super-sickle A′, super-sickle B, and sickle-fibres are characteristic of positions and genotypes of intermediate hairiness. Very strongly medullated fibres, i.e. halo-hairs and hairy-tip–curly-tips, increase in abundance with increasing hairiness of the birthcoat.

The results further confirm the work of Fraser, Ross, and Wright (1954) on the fibre-type shift within (*a*) the pre-curly-tip fibre group, and (*b*) the curly-tip fibres formed by the primary lateral follicles, with increasing *N*-gene dosage. The same shift of fibre types occurs on each of the positions considered individually, but on the different positions the degree of shift with each increase in *N*-gene dosage is not constant.

The average percentage of total pre-curly-tip fibres found in the fibre-type arrays from different positions and genotypes is shown in Table 7.

An analysis of variance between genotypes shows that in non-*N* lambs there are significantly fewer pre-curly-tips on the shoulder patch, shoulder, and back; this may be the result of the pre-natal shedding of baby sickle-fibres (Dry 1933-4) which were found to be present, but it is also possible that, as the abundance of pre-curly-tip fibres becomes lower, primary central follicles grow curly-tip fibres.

Apart from this, there does not appear to be any change in pre-curly-tip fibre abundance between the different genotypes. Between positions, except for the shoulder patch of non-*N* lambs, the most noticeable feature is the low abundance of pre-curly-tips on the withers and neck.

The abundance of total pre-curly-tips has been studied in the New Zealand Romney by Galpin (1936*b*). She grouped the positions, in order of diminishing count, as follows:

Poll	A area
Ventral neck	B area
Shoulder point	C area
Withers, fifth rib, and britch	D area
Side and back	E area
Superior ilium, thurl, and superior flank	F area

Galpin's theory to explain variations in pre-curly-tip abundance was that, on those regions of the foetus where follicles and fibres appear early, there is a greater proportion of pre-curly-tips. This is supported by the work of Galpin (1935), Carter and Hardy (1947), and Wildman (1932), but in view of the discrepancy of results it is suggested that the matter should be re-investigated in both *N*-type and non-*N*.

TABLE 7

DIFFERENCES IN PRE-CURLY-TIP FIBRE ABUNDANCE BETWEEN POSITIONS AND GENOTYPES

Means are transformed fibre percentages using Bliss's angle transformation

Positions	Genotypes					F Values	d
	+/+	*N*/+	*N*/+ . +/*nr*	*nr*/*nr*	*N*/*N*		
Withers	9.97	11.43	12.41	12.00	11.88	$F = 1.15$ $n_1 = 4, n_2 = 27$	
Neck	10.85	12.61	—	14.42	14.02	$F = 1.36$ $n_1 = 3, n_2 = 23$	
Shoulder	11.51	16.86	—	18.73	16.43	$F = 5.56^{**}$ $n_1 = 3, n_2 = 23$	5% level = 3.36 1% level = 4.56
Shoulder patch	6.24	16.35	—	16.73	16.31	$F = 16.08^{**}$ $n_1 = 3, n_2 = 22$	5% level = 3.43 1% level = 4.66
Side	14.22	15.69	16.27	16.89	15.73	$F = 1.05$ $n_1 = 4, n_2 = 26$	
Back	11.35	16.23	16.60	17.96	15.85	$F = 4.28^{**}$ $n_1 = 4, n_2 = 27$	5% level = 3.09 1% level = 4.17
Britch	15.44	17.20	17.81	—	17.79	$F = 1.23$ $n_1 = 3, n_2 = 27$	
F values	$F = 4.72^{**}$ $n_1 = 6$ $n_2 = 21$	$F = 8.42^{**}$ $n_1 = 6$ $n_2 = 77$	$F = 7.12^{**}$ $n_1 = 3$ $n_2 = 14$	$F = 6.45^{**}$ $n_1 = 5$ $n_2 = 14$	$F = 6.05^{**}$ $n_1 = 6$ $n_2 = 35$		
d	5% level = 4.03 1% level = 5.49	5% level = 2.18 1% level = 2.89	5% level = 2.73 1% level = 3.80	5% level = 2.92 1% level = 3.96	5% level = 1.25 1% level = 3.01		

IV. Discussion

The main points arising from the work are that:

(i) In individual lambs there is a gradient in hairiness from the shoulder patch to the britch. This is similar to the gradient in fibre-type arrays found by Galpin (1936*a*) in work on the New Zealand Romney lamb.

(ii) Increases in hairiness with increasing *N*-gene dosage conform to this gradient.

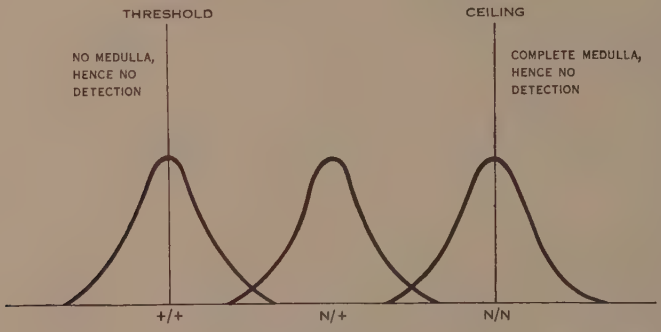

Fig. 9.—Effect of threshold and ceiling on the variation within a genotype.

(iii) With any single increase in *N*-gene dosage there is a differential rate of increase in hairiness on the different positions.

(iv) This differential rate of increase in hairiness can be explained on the hypothesis that the gradient is modified by a threshold and possibly by an approach to a ceiling effect in the production of birthcoat medullation.

The increase in the gradient in hairiness over the body and the variable expression of the phenotype seen in lambs of the *N*/+, *nr*/*nr*, and *N*/+ . +/*nr* group of genotypes can be explained in terms of threshold and ceiling effects. Any variability in the +/+ and *N*/*N* phenotypes, where hairiness is relatively either high or very low, will tend to be buffered by these effects. With intermediate hairiness genetic and environmental variation can reach full expression. The gradient in hairiness, which is measured as variation between different positions, will, similarly, only reach its maximum expression in the absence of any threshold and ceiling effects. The uncertainty of heterozygous expression is well known and has been discussed at length by Goldschmidt (1934, 1938). The effect of threshold and ceiling on the variation in a genotype is shown diagrammatically in Figure 9.

The gradient in hairiness, combined with threshold and ceiling effects, can also explain the different degrees of dominance of the dominant *N*

gene in different positions. Threshold and ceiling effects differ with the use of different methods and the degree of dominance shows parallel variation.

The dominant *N* gene shows either partial dominance (medullometer test results) or else complete dominance in most lambs (Dry's halo-hair studies) on the back, side, and britch positions. There is intermediate phenotypic expression of the heterozygote, i.e. little or no dominance on the shoulder, neck, and withers, and the gene shows either no dominance or some degree of recessiveness on the shoulder patch.

The intensity of the threshold and ceiling effects is a measure of the degree of dominance shown by the gene. In the absence of these effects, and provided there are no genic interactions, increases in hairiness should conform to the gradient and be linear with increases in gene dosage. On any position increases in the threshold effect will lead to recessive phenotypic expression and increases in the ceiling effect will lead to dominance. The combination of a threshold and ceiling effect with a gradient in hairiness over the body leads to the changes in dominance of the dominant *N* gene in different positions. Similarly the different ceiling effects given by the three methods lead to different degrees of dominance of this gene.

The ceiling effect is seen to be partly due to the inability of the method to detect differences when hairiness is high, and there may also be a limit to follicle and hence fibre size. The threshold effect may similarly be caused by inability to detect differences when hairiness is low, as fibres are usually non-medullated below a certain diameter.

There is thus no indication that threshold and ceiling effects are ultimately manifestations of the *N* genes; and the changes in dominance over the body, together with the differential increases in hairiness on different positions, suggest that different *N*-gene dosages may show a linear primary reaction.

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Explanation of Plates 1 and 2

Plate 1

Fig. 1.—Lamb showing halo-hairs.

Fig. 2.—Lamb showing sampling positions. 1, Shoulder patch; 2, shoulder; 3, neck; 4, withers; 5, side; 6, back; 7, britch.

Plate 2

Series of skins showing increasing coverage of the body by grade VII abundance of halo-hairs. Fig. 1.—Grade VI. Fig. 2.—Grade VII*b*. Fig. 3.—Grade VII*c*. Fig. 4.—Grade VII*d*. Fig. 5.—Grade VII*f*.

GENE DOSAGE IN *N*-TYPE SHEEP

1

2

Under these conditions the heat absorbed by the animal due to solar radiation may become overwhelming. Lee (1953) has realized that the test then becomes a measure of the time taken by the animal to lose control of temperature regulation, and is not necessarily a measure of its adaptive ability. Phillips (1948, 1950, 1953) has emphasized the need to develop a measure of the "thermal balancing capacity" in cattle.

An animal's ability to maintain a normal body temperature is determined in a hot environment by its ability to dissipate excess heat. The purpose of this paper is to describe a comparative test which indicates the animal's ability to dissipate heat.

II. Material and Methods

Animals and environment.—The cattle used in this investigation were drawn from a herd of 300 Shorthorns run on natural pastures under

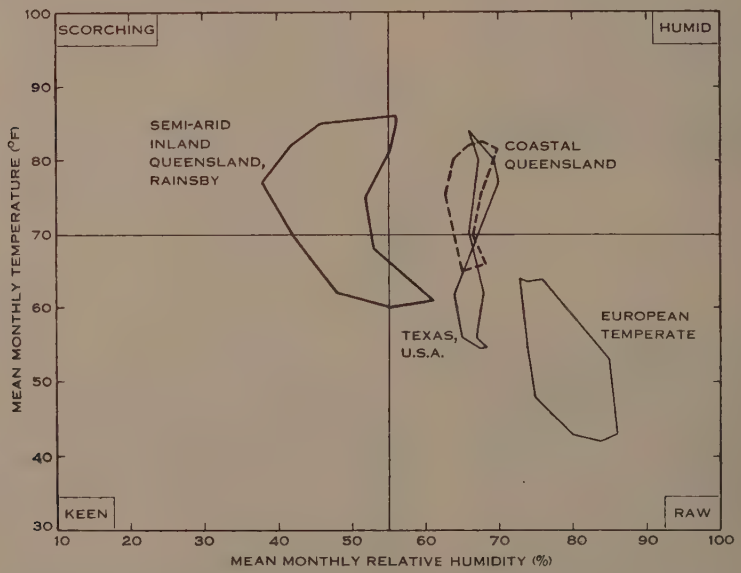

Fig. 1.—Climograph to illustrate the difference in climate between the hot semi-arid inland and the wetter, more humid or coastal regions.

station conditions at Rainsby (21° 9′ S., 145° 1′ E.) in the semi-arid Mitchell grass country of inland Queensland. The sun temperature is over 140°F throughout the year. A climograph of Rainsby is given in Figure 1. This compares Rainsby conditions with those of Texas, U.S.A., of coastal Queensland, and of temperate European areas.

Figure 2 shows graphically differences in rainfall temperature and relative humidity between Rainsby and Rockhampton (23° 23′ S., 150° 31′ E.).

Atmospheric temperatures.—These were shade temperatures taken in an adequate screen. Relative humidity was estimated from three identical readings of wet-bulb and dry-bulb thermometers on a sling psychrometer taken under temporary shade on the cattle yard site.

Management.—Management at Rainsby was simplified by restricting calving to 2 months in the autumn. Calves were tattooed and ear-tagged at birth. Forty bulls were used in this investigation. They were divided

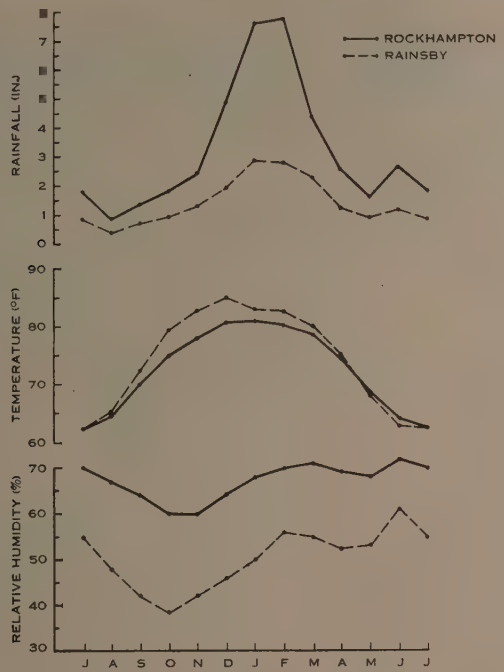

Fig. 2.—Mean monthly rainfall, shade temperature, and relative humidity for Rainsby and Rockhampton, Qld. The mean daily temperatures are misleading. It is much hotter at Rainsby but there is less daily variation in the temperature at Rockhampton.

into three groups. Group A was a group of 10 Australian Illawarra Shorthorns; groups B and C were both pedigree beef Shorthorns. B and C were separated according to their coat characters, 20 animals falling into group B and 10 into group C. The separation does not reflect differences in breeding. It does distinguish, however, differences in growth up to 2 years. (The differences between the groups are described below.)

Classification of the coat.—This was based on hair samples clipped from specific areas. The samples were dried and weighed. The clipped area was measured with callipers, and the weight of hair expressed in milligrams per square centimetre of skin. The degree of medullation was

estimated by calculating the percentage of fibres showing medullation, and the ratio of the diameter of the medulla to the diameter of the fibre in completely medullated fibres.

Body temperatures.—These were taken per rectum with a direct-reading veterinary-thermometer bridge designed and constructed at the National Standards Laboratory, C.S.I.R.O. (Fig. 3). The thermometer was inserted 6-8 in. The speed of response was 10-20 sec.

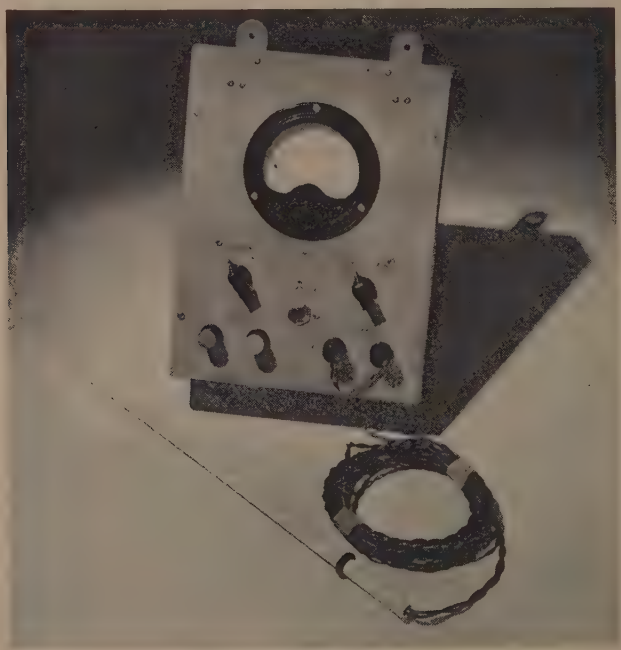

Fig. 3.—Direct-reading veterinary-thermometer bridge.

Treatments.—These consisted of standing animals in the shade, in the sun, or in the shade after exercise, and were applied after first accustoming animals to being handled in the yards. Animals were rested in the yards overnight before each experiment to detect sick or feverish beasts. When exercise was used to raise body temperature the amount had to be adjusted to the environmental temperature, less exercise being required on hot days to raise body temperature to about 104°F. It is not advisable to test the rate of cooling after exercise when the shade temperature is over 95°F, because it is very easy to push cattle too far under these conditions. A feature of this test is that it can be applied quickly and does not require very hot atmospheric temperatures in a clear dry environment. It should be applied early in the morning when very hot conditions prevail.

Skin moisture.—A colour indicator powder, bromophenol blue, was used to detect skin moisture. It was used because it is highly soluble in water (0.4 g/100 ml) and gives a distinct purple coloration when dissolved. The closely clipped area on the animal to be examined was cleansed with alcohol and ether, and the powder dusted on and covered with stopcock grease.

III. Results

Table 1 summarizes a description of the coat characters of the three groups, A, B, and C, and Figure 4 shows histograms of the lengths of samples taken from the three groups.

Table 1

MEAN VALUES OF COAT CHARACTERS OF THE SHORTHORN BULLS USED IN THE HEAT TOLERANCE TEST AT RAINSBY, 1953

Group	Number of Bulls	Weight of Hair Coat (mg/cm²)	Average Dia. of Fibre (μ)	Average Length of Hair Fibres (cm)
A	10	13.7 ± 2.9	61.4 ± 6.7	0.48 ± 0.16
B	20	5.3 ± 1.2	54.3 ± 5.2	0.47 ± 0.21
C	10	21.2 ± 4.8	41.2 ± 6.1	1.57 ± 0.82

Group	Fibres Medullated (%)	Fibres Continuously Medullated (%)	Ratio of Diameter of Medulla to Diameter of Fibre	Felting Grade*
A	97	50	0.35	0
B	70	26	0.26	0
C	4	1.5	0.18	5

* Grade 0 represents complete absence of felting, grade 5 maximum felting.

It will be noticed that group B had a smaller weight of hair per unit area than the other two groups, and that A had a somewhat smaller weight of hair than C. On the other hand, A had the same length of hair as B, the hairs of A being more strongly medullated than those of B. The hairs of C were much longer than those of A and B and were hardly medullated at all. By applying Bonsma's (1949) felting test it was found that felting was confined entirely to the rough coat, characterized by length, fineness, and non-medullation of the hair. The felting grade was classified from 0 (no felting) to 5 (complete felting). The three groups

fall clearly into two classes, A and B having no felting and C felting completely.

The hair samples examined were taken late in summer when the bulls were 2 years old. The differences between B and C can be attributed to differences in the degree to which the winter coat had been shed and replaced by a summer coat. In group A part of the increase in hair

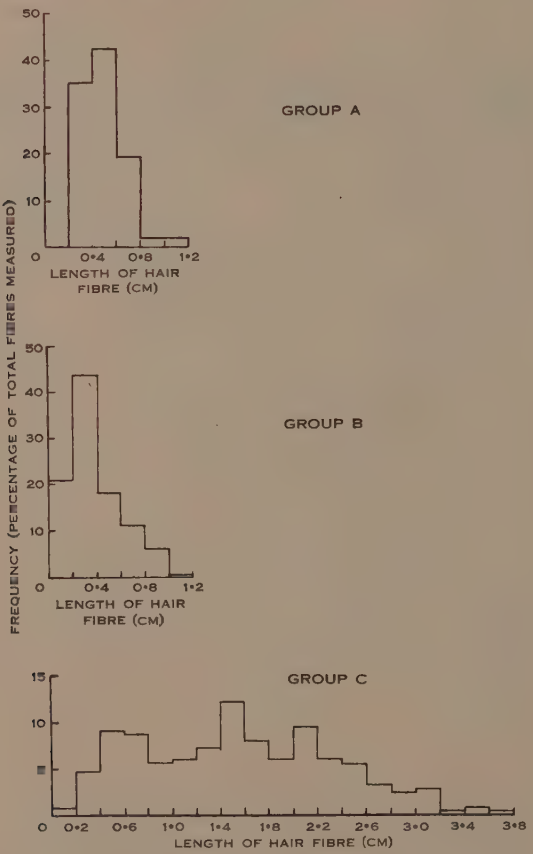
FREQUENCY (PERCENTAGE OF TOTAL FIBRES MEASURED)
GROUP A
GROUP B
GROUP C
LENGTH OF HAIR FIBRE (CM)
0 0·4 0·8 1·2
0 0·2 0·6 1·0 1·4 1·8 2·2 2·6 3·0 3·4 3·8

Fig. 4.—Distribution of hair lengths for groups A, B, and C type coats in Shorthorns.

weight may be a reflection of the promotion of growth of the body as a whole following a quicker response by the animals to improved nutritional conditions. Group B which had shed the winter coat decisively was better developed than C. The average liveweight of B at 2 years was 1030 ± 60 lb, and that of C, which still carried a winter coat though kept under the same conditions as B, was 801 ± 68 lb. The liveweight of A was 1040 ± 33 lb.

In Figure 5, changes in the rectal temperatures of the groups are plotted against the shade temperature. These experiments were all carried out in late summer at the time the hair samples were collected. In this experiment bulls were stood in the shade for a day after settling down as described above. Each curve is the mean of the temperatures of the bulls forming the group represented by the curve. Group A showed little change in rectal temperature. Both B and C showed a tendency for rectal temperature to rise when shade temperatures exceeded 95°F. At all shade temperatures from 70°F upward the rectal temperatures of the groups differed, A being the lowest and C the highest.

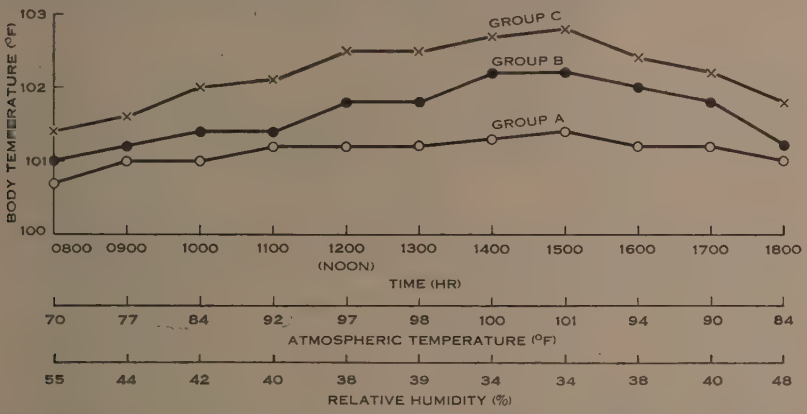

Fig. 5.—Diurnal variation in body temperature in the shade of Shorthorns with different coat types. Group A, high medullation (10 animals); group B, medium medullation (20); group C, no medullation (10).

Figure 6 is a similar set of readings taken when the bulls were standing in the sun. Although shade temperatures were lower on this day, rectal temperatures rose much more noticeably. The groups still retained the same relative order but there was a marked difference between C and the other two groups. Group C reached a rectal temperature of 105°F when shade temperature rose to 97°F. In Figure 7 a further set of readings is shown. These were taken with bulls standing in the sun on a hot day. The experiment was terminated when the rectal temperatures of C reached 107°F. Once more the rectal temperatures of groups A and B stayed fairly close together except when shade temperatures exceeded 100°F, then the rectal temperatures of B started to rise much faster than those of A.

In Figure 8 two experiments are recorded in which groups B and C only were used. The bulls were exercised gently for half an hour on two days, during one of which shade temperatures reached a maximum of 95°F, and during the other, 85°F. The difference between the two groups was most marked. Bulls of group B retained good control of their

body temperature despite a rise to about 103°F. Rectal temperatures did not rise beyond this initial point, and fell off steadily from then on. Bulls of group C, however, were clearly pushed nearly to their physiological limits. Temperatures continued to rise 2 hr after exercise had ceased and remained without falling at a high level for 2 or 3 hr. The differences in temperature between the two groups in this test were as great as those resulting from standing in the sun on a hot day, and can be produced independently of weather conditions. Further, results became available

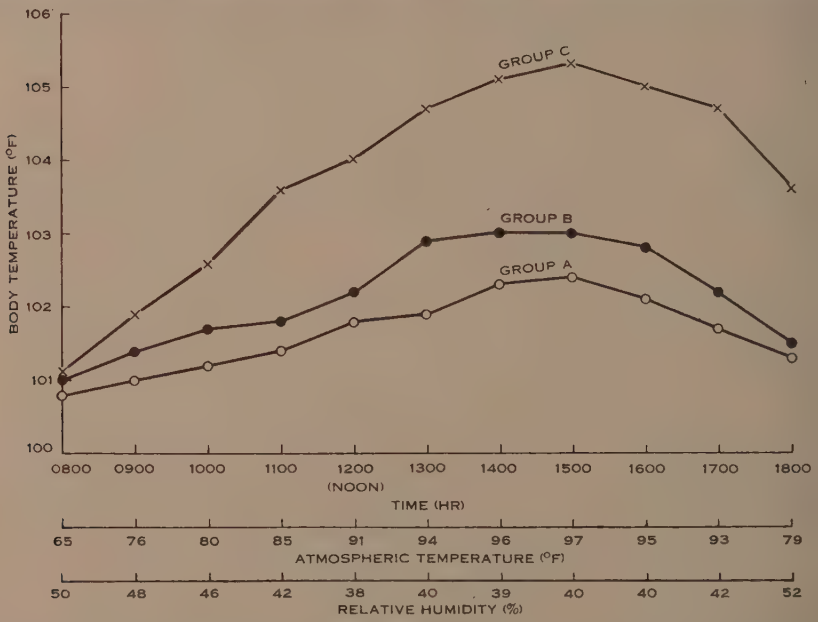

Fig. 6.—Diurnal variation in body temperature in the sun of Shorthorns with different coat types. Group A, high medullation (10 animals); group B, medium medullation (20); group C, no medullation (10).

in a few hours. The test clearly discriminates between animals tested in the same experiment but it is not clear how the results could be given a numerical value; more experience is required before an index analogous to the Iberia index can be devised. However, an analysis of variance of the temperatures on which Figure 8 was based shows that, within wide limits of shade temperature, tests made on different days are directly comparable unless very small differences are being looked for.

An analysis of variance of actual temperatures is shown in Table 2. This analysis shows that by far the greater portion of the variance is due to differences between the groups. These clearly had been chosen so that they contained animals of very different powers of temperature control. The variance due to differences between animals of a group is small.

The groups × days interaction is significant ($P < 0.001$) owing to the differences between group-day means; i.e. the difference (group C − group B) at 85°F is 2.62 and the difference (group C − group B) at 95°F is 2.15. This indicates that actual temperatures cannot be used to compare animals tested on different days without introducing some error.

However, if the initial resting or pre-exercise temperatures are subtracted from the recordings made at times after exercise (Table 3), and an analysis of variance made of these differences, the (groups × days)

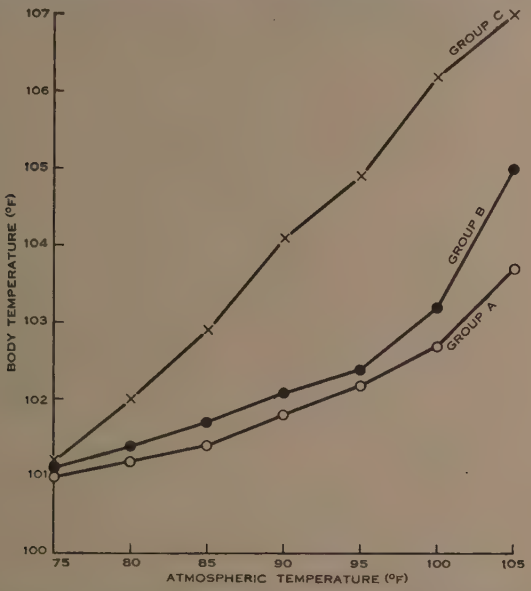

Fig. 7.—Effect on the body temperature of Shorthorns of the absorption of heat due to intense solar radiation. Group A, high medullation (10 animals); group B, medium medullation (20); group C, no medullation (10).

interaction is no longer found to be significant (Table 4): tests made on different days, provided conditions are not more extreme than those reported here, may be considered comparable.

The maximum difference between groups is shown to have occurred approximately 3 hr after the initial reading by which time group B had returned to normal body temperature.

Sweating.—Experiments were undertaken to determine the presence of skin moisture. A colour indicator powder (bromophenol blue) was dusted onto the cleansed skin. There was no evidence of any moisture at 98°F dry-bulb temperature and relative humidity 39 per cent. When stopcock grease was applied over the powdered area and the animals were

exercised, re-examination of the powdered area revealed visible evidence of moisture. In the laboratory this phenomenon of visible moisture loss from the skin under heat stress is readily seen to be due to the function of the apocrine gland, and has been reported (Ferguson and Dowling 1955). The fact that the indicator does not record moisture unless covered with grease shows that the rate of evaporation is rapid, which may account for the doubts which have been expressed in the past about the ability of cattle to sweat.

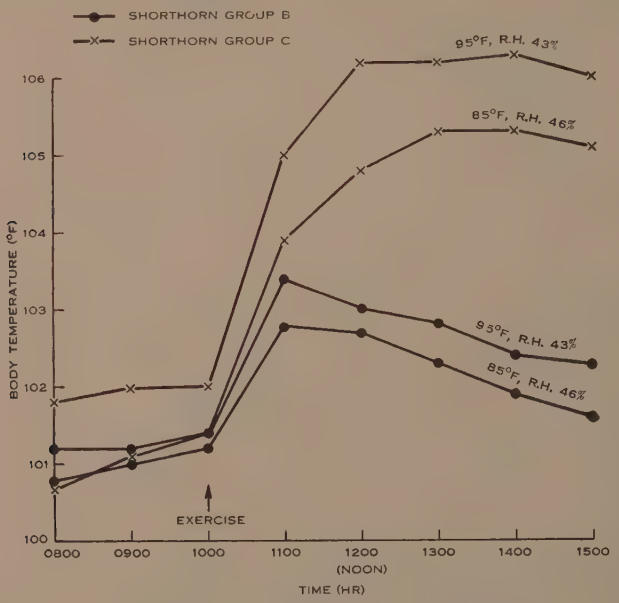

Fig. 8.—Variation in the response of Shorthorns to the Heat Tolerance Test at Rainsby, under two sets of atmospheric conditions. Exercise continued for ½ hr in experiments 1 and 2. Group B, high medullation (20 animals); group C, no medullation (10 animals).

IV. Discussion

The response of all groups to standing in the shade and in high sun temperatures without exercise was similar. In all there was some response to changes in shade temperature, and a very marked one to such changes when the animals were subjected to high sun temperatures. This indicates that large amounts of heat were being absorbed by animals in the sun, and the picture given by Figure 7 is a resultant between absorption of heat, which was going on all the time, and heat dissipation by mechanisms, whatever they may be, which were operating to return body temperatures to normal. Thus the picture is one of a complex of events. By standing animals in the shade the effect of absorption of solar radiation was

eliminated but this reduced the heat load on the animal to a point where differences between groups were much less obvious. When animals were exercised first and then stood in the shade, a heat load was imposed and then removed, leaving the animal's heat-dissipating mechanisms to get

TABLE 2

ANALYSIS OF VARIANCE OF BODY TEMPERATURE OBSERVATIONS IN HEAT TOLERANCE TEST

Source of Variance	Degrees of Freedom	Mean Square	*F*	*P*
Times	4	3.0	27.2	<0.001
Days	1	37.25	109.55	<0.001
Days × times	4	0.07	17.5	<0.001
Between groups	1	553.7	512.7	<0.001
Between animals within groups	28	1.08		
Groups × times	4	12.07	109.7	<0.001
Between animals within groups × times	112	0.11		
Groups × days	1	4.78	14.05	<0.001
Between animals within groups × days	28	0.34		
Groups × times × days	4	0.48	120	<0.001
Between animals within groups × times × days	112	0.004		
Total	299			

rid of excess heat without the complication of further heat loading due to solar radiation. The differences between groups B and C after exercise may therefore be taken to represent differences in cooling efficiency.

TABLE 3

MEANS AND STANDARD ERRORS OF DIFFERENCE IN BODY TEMPERATURE BETWEEN TIMES AND DAYS WHEN THE INITIAL PRE-EXERCISE (RESTING) TEMPERATURE IS SUBTRACTED

Group	Day 1					Day 2				
	Time 1	Time 2	Time 3	Time 4	Time 5	Time 1	Time 2	Time 3	Time 4	Time 5
B	1.83 ±0.05	1.7 ±0.08	1.5 ±0.1	0.9 ±0.1	0.60 ±0.07	2.24 ±0.07	1.83 ±0.06	1.63 ±0.06	1.23 ±0.088	1.19 ±0.08
C	2.8 ±0.09	3.8 ±0.11	4.3 ±0.17	4.3 ±0.16	4.1 ±0.17	3.0 ±0.12	4.2 ±0.13	4.2 ±0.07	4.3 ±0.1	4.0 ±0.1

Observations of the rate of cooling of an animal after exercising can be taken as a better indication of ability to dissipate heat than measurement of temperature with the animal standing in the sun or in the shade without prior exercise. The distinction is perhaps less important in this series of experiments, in which all animals were similar, than it would

be if comparisons were made between groups with very different coat colours in which the heat absorption in sunlight would be very different (Stewart 1953). This illustrates the basic weakness of any test in which several uncontrolled variables are present at the same time.

The differences in temperatures between the groups was associated with coat character. Groups A and B were not very different in their temperatures throughout the series of experiments, A being better at controlling temperature than B, so that if we are to look for an explanation

TABLE 4

ANALYSIS OF VARIANCE OF DIFFERENCES IN BODY TEMPERATURE OBSERVATIONS IN HEAT TOLERANCE TEST WHEN THE PRE-EXERCISE BODY TEMPERATURE IS SUBTRACTED

Source of Variance	Degrees of Freedom	Mean Square	*F*	*P*
Times	4	2.927	50.46	<0.001
Days	1	5.44	10.52	<0.001
Times × days	4	0.09	4.5	
Between groups	1	401.24	703.9	<0.001
Between animals within groups	28	0.57		
Groups × times	4	12.76	212.6	<0.001
Between animals within groups × times	112	0.058		
Groups × days	1	1.84	3.54	N.S.
Between animals within groups × days	28	0.517		
Groups × times × days	4			
Between animals within groups × times × days	112	0.02		
Total	299			

of the difference in heat control between A and B, on the one hand, and C, on the other, in the differences of coat quality, clearly it must lie in the length and medullation of the fibres of the coat and not in its total weight. The group A coat is nearer in weight to the C than to the B coat, but both B and A have similar lengths and medullations whereas C coats are longer, finer, non-medullated, and felt.

In the light of the experiments of Yeates (1955) it seems reasonable to attribute some of the differences in body temperature we found here to differences in coat cover. These might be acting either as a straightforward insulator, or by interfering with the cooling by the evaporation of moisture. Yeates found that the difference in temperature between an animal in full winter coat and one in full summer coat was 1.5°F at 105°F in the hot room. This does not seem nearly enough to account for the differences found here.

However, it is known that cattle sweat (Ferguson and Dowling 1955), and it is not possible to say to what extent differences between

the groups may have been due to differences in sweat production. The sweat production of the bulls used in this experiment has not been measured. It is known, however, from subsequent work, which will be described in a separate paper, that Australian Illawarra Shorthorns have a higher sweat-gland volume per unit area of skin than beef Shorthorns, which may account for the difference between A and B, and that animals which have not shed their winter coat, such as group C, have sweat-gland volumes per unit area which may be as low as 60 per cent. of that found in animals typical of group B. Thus the difference in the groups as far as temperature tolerance goes may in part be due to differences in sweating, and this difference in temperature tolerance may, through the condition of the animal, be the cause of coat differences, the cattle which do not thrive failing to shed their coat. As far as the data reported here go, it is not possible to distinguish between an interpretation which supposes that the long hair of group C is responsible for lack of heat tolerance and the one which supposes that lack of heat adaptation is responsible for a long coat. It may be mentioned that animals of group C reach temperatures of 107°F but cool rapidly when sprayed, and if sprayed with warm water before exercise will show temperatures following curves typical of group B. These observations were made apart from the experiments reported here.

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SWEAT GLANDS IN ZEBU (*BOS INDICUS* L.) AND EUROPEAN (*B. TAURUS* L.) CATTLE

I. SIZE OF INDIVIDUAL GLANDS, THE DENSENESS OF THEIR POPULATION, AND THEIR DEPTH BELOW THE SKIN SURFACE

By T. NAY* and R. H. HAYMAN†

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Summary

Number of sweat glands per unit area of skin, their size, and their depth below the epidermis were compared in skin biopsy specimens obtained from the midside region of two Zebu and three European breeds of dairy cattle. Within Zebu breeds, comparisons were made between sweat glands from the midside and the dewlap.

Zebus were found to have much larger and more numerous sweat glands than European animals. Within Zebus, sweat glands were slightly larger, and much more numerous, on the midside than on the dewlap. They are much closer to the skin surface in Zebu cattle than in European.

In Zebus sweat glands are longer and of greater diameter than in European cattle. In appearance they are sac-like, with few convolutions, whereas sweat glands in European cattle are rarely sac-like, and quite convoluted.

I. INTRODUCTION

Differences between Zebu and European cattle in size of sweat glands and their number per unit area of skin surface have been indicated by previous workers. So far, attention has been directed mainly toward estimation of population density.

Kelley (1932) mentioned differences between Zebu and Zebu × Friesian crossbreds in the number of sweat glands per unit area of skin, Zebus having the greater number. He also stated that in skin sections from pure-bred Friesian cattle, sweat glands were small and difficult to find. Yamane and Ono (1936) compared numbers of sweat glands per unit area on each of 21 body regions in Zebu cattle, European cattle (Friesians), and Formosan buffaloes. They found Zebus to have the greatest number and Friesians the least. They also measured the length of the largest observable sweat gland at each site. Findlay and Yang (1950) described the morphology of sweat glands and the denseness of their population in several body regions of Ayrshire cattle. Dowling (1955) demonstrated very clear differences between Zebus and beef Shorthorns in numbers of sweat glands per unit area of skin. His techniques were adequate to overcome problems of shrinkage in his material, and his results showed that there were approximately 50 per

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cent. more sweat glands per unit area in Zebus than in Shorthorns. Hafez, Badreldin, and Shafei (1955) discussed sweat glands in Zebu cattle and Egyptian buffaloes (*Bos bubalus*). Zebus were found to have a much higher population of sweat glands per unit area of skin, and the glands themselves were larger.

The purpose of the present paper is to describe, in quantitative fashion, results obtained from a study of sweat gland size, depth of glands below the skin surface, and density of gland population per unit area, in skin biopsy specimens obtained from dairy-type Zebu and European cattle.

II. Animals

European breeds were represented by two Jersey cows and one 2½-year-old heifer, two Red Poll cows, and three Friesian cows. All were running in the dairy herd on the F. D. McMaster Field Station, C.S.I.R.O., near Sydney, N.S.W. Zebu breeds were represented by four 3-year-old Sindhi, and five 3-year-old Sahiwal heifers. These animals had been given to the Commonwealth Scientific and Industrial Research Organization by the Pakistan Government, and were located on the Field Station at Werribee, Vic. All animals, both European and Zebu, were in fat condition.

III. Material and Methods

Biopsy specimens were taken from the midside of each animal of both Zebu and European species. Additional samples were obtained from the dewlaps of the Zebus. Samples were taken with a 1 cm diameter trephine, after the method described by Carter and Dowling (1954), and immediately placed in 5 per cent. formol-saline. Sampling was carried out in June 1955, several samples being taken from each site.

(*a*) *Preparation of Material for Measurement of Sweat Gland Size*

Biopsy specimens were transferred from 5 per cent. formol-saline to 70 per cent. alcohol for 24 hr to harden. Vertical sections were then cut by hand, using an ordinary safety razor blade. The plane of sectioning was kept parallel to the direction in which the hairs grew. The resulting sections were from 300 to 350 μ in thickness, and contained numbers of undamaged sweat glands. Care was taken to prevent the material from drying out during the cutting and staining processes.

Sections were stained in 0.5 per cent. thionin dissolved in 20 per cent. alcohol, which proved an almost specific stain for sweat glands. After staining for 5-10 min, the sections were wetted quickly in water. They were then transferred, for 2-3 min, to a mixture of equal parts of 1 per cent. ferric cyanide and 5 per cent. potassium molybdate, prepared shortly before use. They were washed quickly in water, dehydrated in two changes of absolute alcohol, cleared rapidly in xylol (less than 10 min), and mounted in "Siro".

This method was undertaken so that shrinkage of material would not upset subsequent gland measurements. Its efficiency in reducing shrinkage was tested by measuring sweat glands in sections cut from fresh biopsy specimens, and again measuring the same glands after the sections had been treated as described above.

In the preparation of horizontal skin sections greater shrinkage was observed in sections obtained from European cattle (48 per cent.) than in those from Zebus (33 per cent.). Material from European cattle was, therefore, used to test shrinkage in vertical sections. Much difficulty was experienced in cutting suitable sections from the fresh material. However,

TABLE 1

MEASUREMENTS OF SWEAT GLANDS MADE ON FRESH MATERIAL, AND ON THE SAME GLANDS AFTER HISTOLOGICAL PREPARATION

Measurements in micrometer eyepiece units; 1 unit = 14.8 μ

Gland	Fresh Material		After Preparation	
	Length (eyepiece units)	Diameter (eyepiece units)	Length (eyepiece units)	Diameter (eyepiece units)
1	53	7, 8, 8, 11	55	9, 7, 7, 12
2	57	6, 8, 7	60	6, 8, 7
3	35	8, 7, 3	35	8, 7, 3
4	66	4, 6, 8, 7, 7	64	5, 7, 7, 7, 7
5	43	7, 5, 6	42	7, 5, 6

sections were finally obtained in which glands could be measured in the fresh state. Five glands were measured before and after treatment. Measurements were made along their length from duct to base, and across the widest portion of each separate convolution. The results of the measurements, given in Table 1, indicate that they were little affected by shrinkage.

(*b*) *Preparation of Material for Estimation of Number of Sweat Glands per Unit Area of Skin*

Biopsy specimens, after being fixed in 5 per cent. formol-saline, were placed in 70 per cent. alcohol for 24 hr, after which they were dehydrated for 2½ hr in "Cellosolve". They were then cleared in cedarwood oil (two changes, each of 2½ hr) and embedded in paraffin in a vacuum embedder, again being subjected to two changes, each of 2½ hr.

Transverse sections, each 20 μ thick, were cut with a microtome and stained for 2 min in 0.5 per cent. methylene blue. After staining, the sections were passed rapidly to absolute alcohol, cleared quickly in xylol, and mounted in "Siro". On using this technique, hair follicles and sebaceous glands stained blue, sweat glands stained purple owing to their large nuclei, and connective tissue was virtually unstained.

(c) *Methods of Measurement*

(i) *Measurements of Gland Size.*—Using an eyepiece micrometer scale, one measurement of gland length, from the junction of the gland with the duct to the outside edge at the base, and three of gland width were made, as shown in Figure 1. Glands which had been damaged so as to cause any distortion or collapse were not measured, nor were those which could not be seen in full lateral view (see Fig. 1). In calculating size each gland was treated as a cylinder of length equal to the measured

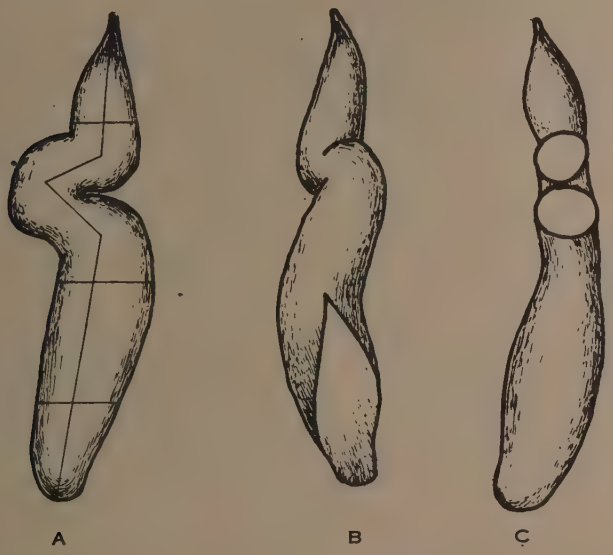

Fig. 1.—*A*, sweat gland visible in full lateral view. Line running from end to end of gland indicates measurements of length. The three lines running across gland represent measurements of width. *B*, damaged sweat gland, not measured. *C*, sweat gland not visible in full lateral view, not measured.

length, and diameter equal to the mean of the three measurements of width. Agreement between measurements made by each of us measuring the same gland independently, and between our own repeated measurements, was found to be excellent. Each of us measured the first 11 clearly observable glands in a section from each site from each Zebu animal and the first 13 from the midside area of each European animal. This resulted in measurements of approximately 200 individual glands from comparable midside regions for each species. The measurements were made independently in the separate laboratories in which each of us worked.

Despite the close agreement between our measurements during preliminary work, we subsequently differed as regards measurements of gland size in European cattle. The differences were due to an error by one of us in measurement of diameter, and his data have therefore been excluded from further consideration.

(ii) *Estimations of Sweat Gland Number.*—These estimations were made on transverse sections, in which shrinkage of the material was an important factor. The area of each section was measured under low power of the microscope, using a 1 sq. cm graticule. The area of the biopsy specimen originally removed by the 1 cm trephine was 0.789 sq. cm. Shrinkage was obtained by subtracting the measured area of the section from its original area. Using the same power, the number of hair follicles within each of 10 adjacent 1 mm squares were counted, the average being corrected for shrinkage and expressed as number of hair follicles per square centimetre of skin area. Follicle counts were easier and quicker to make than sweat gland counts and, as has been amply demonstrated, every hair follicle is accompanied by a sweat gland in cattle (Shiefferdecker 1917; Turley 1926; Yamane and Ono 1936; Findlay and Yang 1950; Barrison Villares and Berthet 1951*a*, 1951*b*; Carter and Dowling 1954).

(iii) *Vertical Distances from the Epidermis to the Junction of the Sweat Gland and Sweat Gland Duct.*—These were determined from the material prepared for estimation of sweat gland size. Measurements were made with the aid of an eyepiece micrometer scale, and the readings converted to microns. Distances were measured for each of the first observable 10 sweat glands in one section from each animal.

IV. Results

(*a*) *Sweat Gland Size within Zebu Breeds*

Data for mean sweat gland size in skin samples from dewlap and midside regions for the individual animals of each Zebu breed are presented in Table 2.

Sahiwals appear to have larger sweat glands on the midside than Sindhis, and within this breed sweat glands on the midside are larger than those on the dewlap. There is no apparent difference between breeds as regards size of sweat glands on the dewlap.

Because of the small numbers of animals within them, data for both breeds were combined and tested for differences between animals, and between positions on the same animal. The resulting analysis of variance, in which a split plot design was used, is given in Table 3.

Differences between animals, and between positions on the same animal, were not statistically significant. The interaction mean square for animals $\times$ positions is of interest, and yields a large F value when tested

with the mean square for observers × positions. This is accepted as reflecting the significance of the difference between sweat gland size on the body as compared with the dewlap, in the Sahiwal breed.

TABLE 2

MEAN SIZE OF 22 SWEAT GLANDS FROM EACH OF TWO BODY REGIONS ON ANIMALS OF TWO ZEBU BREEDS

Animal No.	Breed	Sweat Gland Size		Mean Size for Breed	
		Dewlap ($\mu^3 \times 10^6$)	Midside ($\mu^3 \times 10^6$)	Dewlap ($\mu^3 \times 10^6$)	Midside ($\mu^3 \times 10^6$)
1	Red Sindhi	14.5 ± 3.8	12.7 ± 3.5	20.3 ± 9.1	20.4 ± 6.2
2	Red Sindhi	18.5 ± 5.4	24.6 ± 10.5		
3	Red Sindhi	14.6 ± 7.2	17.8 ± 5.6		
4	Red Sindhi	33.7 ± 10.8	26.0 ± 11.8		
1	Sahiwal	16.8 ± 5.7	35.4 ± 11.8	19.6 ± 2.9	25.4 ± 6.1
2	Sahiwal	18.0 ± 6.3	23.5 ± 9.5		
3	Sahiwal	24.2 ± 11.0	21.6 ± 6.9		
4	Sahiwal	18.7 ± 6.1	26.3 ± 5.9		
5	Sahiwal	20.4 ± 6.8	20.0 ± 8.0		

(*b*) *Sweat Gland Size within European Breeds*

Data for mean sweat gland size in skin samples from the midside region of Jersey, Friesian, and Red Poll cattle are given in Table 4. Analysis of variance shows that differences between breeds are not significant, but that those between animals within a breed are highly so ($F = 5.22^{***}$; D.F. 5, 96).

TABLE 3

ANALYSIS OF VARIANCE: SWEAT GLAND SIZE IN ZEBU CATTLE

Source of Variation	D.F.	Mean Square	*F*
Between animals	8	1047.23	2.68 N.S.
Between positions	1	946.75	2.42 N.S.
Animals × positions*	8	390.43	11.96**
Observers	1	4.74	
Animals × observers	8	48.26	
Positions × observers†	1	32.64	
Residual	368	69.67	
Total	395		

* Error term for testing animals and positions.
† Error term for testing animals × positions.
** $P < 0.01$. N.S., not significant.

(*c*) *Density of Sweat Gland Population in Zebu and European Breeds*

Numbers of sweat glands per square centimetre of skin in the dewlap and midside areas of Zebu cattle, and the midside area from European cattle, are given in Table 5.

Differences between species are apparent when mean numbers for their respective midside areas are compared. The difference is highly significant ($t_{15} = 5.69^{***}$). In Zebu cattle, density of sweat gland population is significantly lower on the dewlap than on the midside

TABLE 4

MEAN SIZE OF 13 SWEAT GLANDS FROM MIDSIDE REGION OF ANIMALS OF THREE EUROPEAN DAIRY BREEDS

Animal No.	Breed	Sweat Gland Size ($\mu^3 \times 10^6$)	Mean Size for Breed ($\mu^3 \times 10^6$)
1	Jersey	10.5 ± 4.9	8.4 ± 2.3
2	Jersey	8.6 ± 1.3	
3	Jersey	6.0 ± 1.5	
1	Friesian	12.4 ± 3.6	10.3 ± 2.0
2	Friesian	8.4 ± 3.9	
3	Friesian	10.1 ± 3.8	
1	Red Poll	10.2 ± 3.9	12.4 ± 3.1
2	Red Poll	14.6 ± 5.0	

($t_{16} = 4.83^{***}$). For comparative purposes photomicrographs of horizontal sections taken at the sweat gland level from Zebu and European cattle are shown in Plate 1, Figures 1 and 2.

TABLE 5

NUMBER OF SWEAT GLANDS PER SQUARE CENTIMETRE ON THE DEWLAP AND MIDSIDE REGION OF ZEBU CATTLE, AND THE MIDSIDE REGION OF EUROPEAN CATTLE

Animal No.	Sindhi		Sahiwal		Jersey	Friesian	Red Poll
	Dewlap (no./cm²)	Side (no./cm²)	Dewlap (no./cm²)	Side (no./cm²)	Side (no./cm²)	Side (no./cm²)	Side (no./cm²)
1	1095	1246	965	1262	1111	963	1029
2	1072	1272	1159	1562	878	1014	934
3	1284	1740	952	1738	1026	1010	
4	1017	1778	1001	1655			
5			1253	1320			
Mean	1117	1509	1066	1507	1005	996	981

(*d*) *Vertical Distance from Epidermis to Junction of Sweat Gland and Sweat Gland Duct in Zebu and European Cattle*

Data for mean measurement of depth of sweat gland below the epidermis for dewlap and midside regions in Zebu cattle and for the midside region in European cattle are shown in Table 6. They indicate a relatively small difference between the Zebu breeds which is not statistically significant ($t_8 = 0.86$; N.S.). In both breeds, but particularly in Sahiwals, sweat glands are further from the epidermis in the dewlap than in the midside.

TABLE 6

DEPTH OF SWEAT GLANDS FROM DEWLAP AND MIDSIDE REGIONS OF TWO BREEDS OF ZEBU CATTLE, AND FROM MIDSIDE REGION OF THREE BREEDS OF EUROPEAN CATTLE

Means of measurements on 10 glands from each region within each breed

Zebu						European			
Animal No.	Breed	Depth to Sweat Gland (μ)		Mean Depth for Breed (μ)		Animal No.	Breed	Depth to Sweat Gland Midside (μ)	Mean Depth for Breed Midside (μ)
		Dewlap	Midside	Dewlap	Midside				
1	Red Sindhi	813 ± 154	836 ± 85	780 ± 37.0	756 ± 72.4	1	Jersey	925 ± 49	940 ± 138.1
2	Red Sindhi	728 ± 72	788 ± 119			2	Jersey	1085 ± 82	
3	Red Sindhi	779 ± 126	669 ± 125			3	Jersey	810 ± 96	
4	Red Sindhi	798 ± 88	729 ± 121						
						1	Friesian	914 ± 96	896 ± 58.0
1	Sahiwal	894 ± 148	662 ± 106	795 ± 88.0	699 ± 67.9	2	Friesian	832 ± 91	
2	Sahiwal	822 ± 101	738 ± 62			3	Friesian	944 ± 66	
3	Sahiwal	853 ± 110	604 ± 72						
4	Sahiwal	691 ± 68	710 ± 90			1	Red Poll	1030 ± 116	1112 ± 116.7
5	Sahiwal	716 ± 78	779 ± 107			2	Red Poll	1195 ± 122	

Results of analysis of variance of the data for Zebus are given in Table 7. Differences between means for animals, and between means for positions, are not statistically significant. The interaction mean square, however, yields a highly significant F value. Again, the interaction is due to differences between positions in the Sahiwal breed.

TABLE 7
ANALYSIS OF VARIANCE: DEPTH OF SWEAT GLANDS IN ZEBU CATTLE

Source of Error	D.F.	Mean Square	F
Animals	8	124.6	N.S.
Positions	1	859.0	N.S.
Animals × positions	8	287.6	4.87***
Error	162	59.0	
Total	179		

*** $P < 0.001$. N.S., not significant.

Differences between breeds in European cattle are indicated in Table 6 but the small numbers do not warrant statistical analysis. However, the data may be compared with those from the midside region of the Zebus.

TABLE 8
ANALYSIS OF VARIANCE: DEPTH TO SWEAT GLANDS ON MIDSIDE REGIONS OF ZEBU AND EUROPEAN CATTLE

Source of Variation	D.F.	Mean Square	F
Error	153	43.65	
Between animals within breeds and species	12	440.66	10.10***
Between breeds within species	3	1030.00	2.34 N.S.
Between species	1	11573.00	11.24*

* $P < 0.05$. *** $P < 0.001$. N.S., not significant.

Results of analysis of variance of the data from European cattle, and data from the midside region of Zebu cattle, are given in Table 8. They show significant differences between species, and between animals within breeds. Differences between breeds within a species, however, are not statistically significant.

(*e*) *Morphology*

Photomicrographs of vertical sections cut from biopsy specimens of both Zebu and European cattle are shown in Plate 2, Figures 1 and 2. The glands from European cattle are generally convoluted, extensively so in many cases, whereas those of Zebu cattle are more sac-like and have few convolutions or none at all. For descriptive purposes mean lengths and mean diameters of sweat glands for the two species are presented in Table 9.

V. Discussion

Brody (1945) suggested that Brahman (Zebu) cattle stand heat better than European cattle because they probably have more sweat glands. However, on the whole he does not regard sweating as an important means of heat loss in cattle. Findlay and Yang (1950), as a result of histological studies on the skin of Ayrshire cattle in Scotland, concluded that sweating in this breed was unimportant as a source of heat loss. Findlay (1950), in reviewing literature up to that time, concluded that there had been no work from which it could be concluded that cattle did sweat. However, Riek and Lee (1948), when making observations on Jersey cows in a psychrometric room, calculated that their observed evaporative loss could not be adequately explained unless they considered sweating to be a factor as well as respiratory transpiration. Knapp and Robinson (1954) concluded that a Jersey cow under hot-room conditions efficiently maintained

Table 9

MEAN DIMENSIONS OF ALL SWEAT GLANDS MEASURED IN ZEBU AND EUROPEAN CATTLE

Dimension	Zebu			European	
	No. of Observations	Dewlap	Midside	No. of Observations	Midside
Mean length (μ)	198	914	936	104	724
Mean diameter (μ)	198	169	173	104	129

a heat balance by a good sweating mechanism. Klemm and Robinson (1955) stated that in two Zebu × Hereford calves, sweat glands became active at a skin temperature of approximately 96°F. With the onset of sweating a fall in skin temperature was observed. Ferguson and Dowling (1955) measured sweat production in a Zebu × Jersey heifer and in an Ayrshire heifer. The body weights of the two animals were 360 and 207 lb respectively. After 3 hours' exposure at an air temperature of 118°F and a relative humidity of 9 per cent., the crossbred heifer was producing sweat at the rate of 620 g/sq. m/hr; whereas sweat production in the Ayrshire heifer was at the rate of 140 g/sq. m/hr.

Our present data are concerned with the sweat gland as a potential source of moisture loss. Comparisons of sweat gland size, number, and position are therefore of importance. Mean volume of all glands measured from the midside of the Zebu cattle was $23.1 \times 10^6\ \mu^3$, compared with a mean of $10.1 \times 10^6\ \mu^3$ for similarly situated sweat glands in European cattle. Mean number of sweat glands on the midside region was 1508 per sq. cm of skin surface for Zebus and 996 per sq. cm for European animals. The Zebu animals thus had sweat glands on the midside which were two and a half times as large and one and a half times as numerous as

those in European animals. For these reasons Zebus must be considered to have a far greater potential for moisture loss by sweating than European cattle.

As between breeds within each species there is a greater range in sweat gland size on the midside of the body in European cattle than in Zebus. However, for animals within a breed, the extent of the range in gland size is much greater in Zebus. The extent to which the ranges overlap (Tables 2 and 4; Fig. 2) suggest that selection for sweat gland size might be used to raise heat tolerance in European cattle.

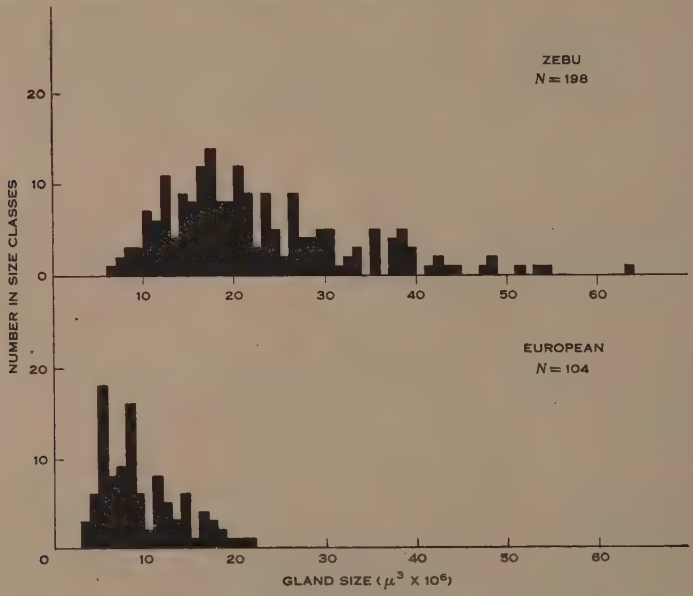

Fig. 2.—Frequency distribution of sweat gland size in Zebu and European cattle.

Numbers of animals within the breeds used in this study are small, and statistical examination of the data for sweat gland size does not indicate any significant difference between them. However, it is interesting to observe that the breeds in each species which have the largest animals also have the largest mean sweat gland size on the midside region. The relevant material is shown in Table 10, in which the breeds in each species are ranked according to body size and mean sweat gland size.

Using our technique Nay and Dowling (personal communication) have shown that beef Shorthorns, although very large cattle, have very much smaller sweat glands than any of the breeds under discussion here. Possibly in this instance the difference in size betweeen beef and dairy types may be associated with the higher metabolic rate of dairy cattle and their consequent need to dissipate more heat.

Densities of sweat gland populations from midside areas for the two Zebu breeds are similar to figures quoted by Dowling (1955) for beef-type Zebus in Queensland, which are probably of Guzerat origin. In the case of European cattle, our data for Jerseys agree with those of Carter and Dowling (1954), but our figures for Friesian and Red Polls appear somewhat greater. Thus it would seem that there is a greater range in density of sweat gland population among European breeds than is the case with Zebu breeds, although so far there is no evidence that any European breed has as many sweat glands as the latter species.

TABLE 10

BODY SIZE AND SWEAT GLAND SIZE IN ZEBU AND EUROPEAN DAIRY BREEDS OF CATTLE

Species	Breed	Rank According to Body Size	Rank According to Sweat Gland Size at Midside
Zebu	Sahiwal	1	1
	Sindhi	2	2
European	Red Poll	1	1
	Friesian	2	2
	Jersey	3	3

The lower population density of sweat glands on the dewlap of Zebu cattle relative to the midside region and, in the Sahiwal breed, their smaller size, suggests that the function of this appendage is not so much one of heat loss by direct sweating, but may be more important from the point of view of evaporation. Thus, sweat trickling from the neck down onto the dewlap will vaporize there and so cool the peripheral blood-vessels. A similar function may be postulated for the extensive umbilical skin fold which is also present in Zebus.

The shorter distance between the epidermis and the upper portion of the sweat gland in the Zebu, as compared with the European species, is difficult to explain.

VI. ACKNOWLEDGMENTS

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Explanation of Plates 1 and 2

Plate 1

Photomicrographs of horizontal sections of cattle skin showing sweat glands in cross section. × 82.

Fig. 1.—European. Fig. 2.—Zebu.

Thick sections cut by hand at about the mid level of the sweat glands, and prepared as described in Section III(*a*). The sections were cut at a sufficient thickness to show (Fig. 1) some of the convolutions typical of European cattle.

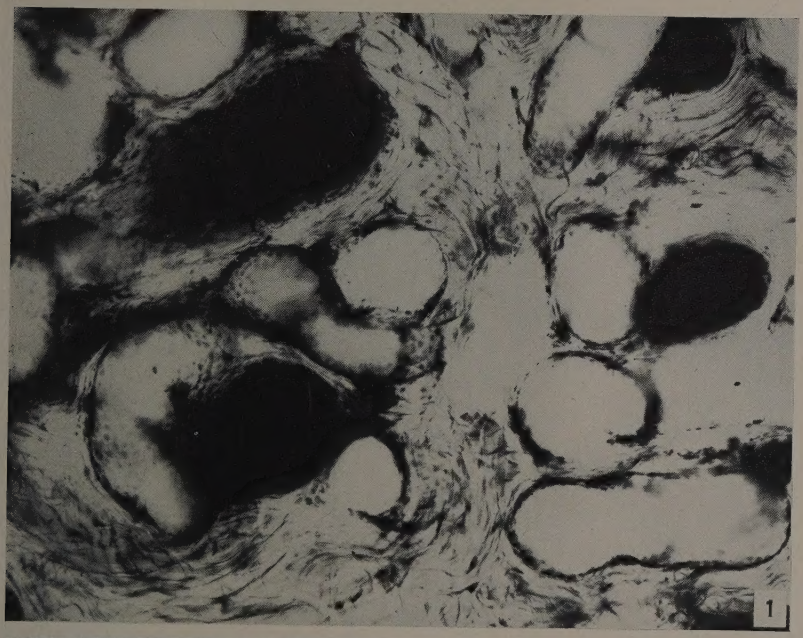
Plate 2

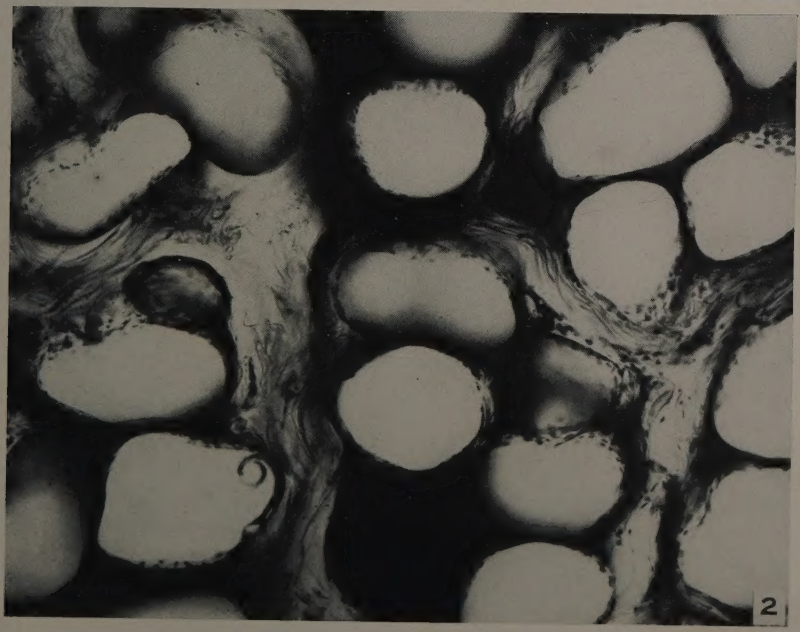
Photomicrographs of vertical sections of cattle skin showing sweat glands in lateral view. Preparation as described in Section III(*a*). × 74.

Fig. 1.—European. Fig. 2.—Zebu.

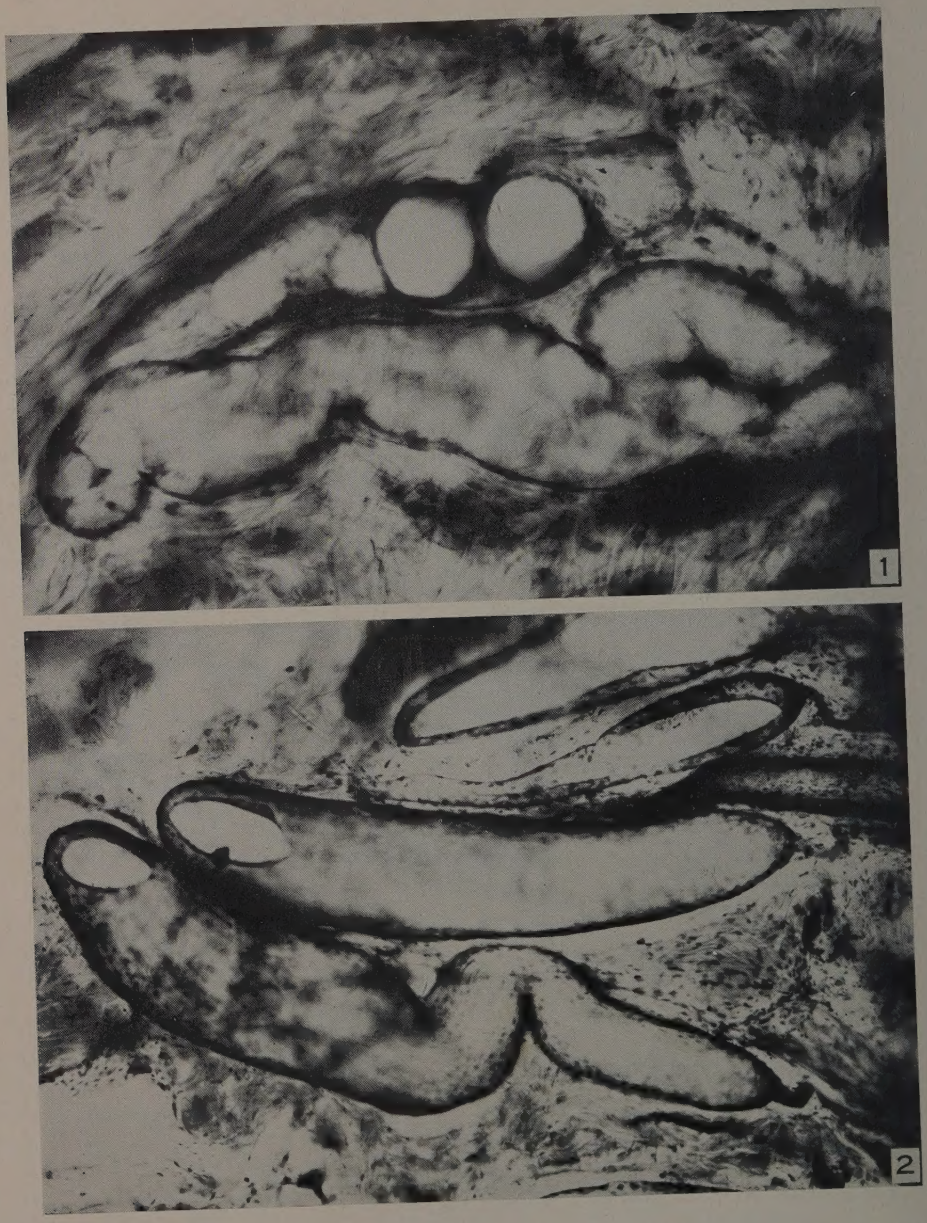
The difference in degree of convolution in glands from European and Zebu cattle is quite clear. Owing to the difficulty in photographing such thick sections, it was not possible to find a sweat gland in the material from European cattle which could be photographed as a typical specimen for both size and shape. The gland which was photographed is an unusually large one.

SWEAT GLANDS IN CATTLE. I





SWEAT GLANDS IN CATTLE. I



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